

nature

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Take your time, Mr Benn

LIKE a good thriller, the Royal Commission on Environmental Pollution's report on the environmental risks of nuclear power, published last week, gives relatively little away in its early chapters. With the greatest of care (and surely such a readable report deserves a wider audience than the Stationery Office is accustomed to delivering) the scene is set in what could serve as an intelligent layman's guide to nuclear energy. A little pat on the back here, a modest rebuke there; a new committee suggested, a possible gap in interdepartmental responsibilities pointed out. Then, with the end of the book almost in sight the action quickens—in a somewhat unpredictable way—and the final pages are highly controversial.

The commission, chaired for the duration of the preparation of this report by Sir Brian Flowers (though now headed by Professor Hans Kornberg), has as its remit to advise on environmental pollution, on research, and on future dangers to the environment. The projected growth of the civil nuclear power programme, with all the associated general concern over possible radiological hazards, stands out as an appropriate field of study for the commission, particularly with pressures growing for an early government decision on major investment in a demonstration commercial fast-breeder reactor (CFR). The report has come none too soon; authorisation to go ahead with the design for the first CFR—popularly known as CFR-1—was being sought from the Energy Secretary, Mr Benn, by the United Kingdom Atomic Energy Authority (UKAEA) within a matter of weeks. At least the publication of the report will give Mr Benn good cause to delay his decision. Careful analysis of the commission's arguments could conceivably persuade him that clearance for CFR-1 would be an unwise option. For the widespread im-

plications of such commitment, even to just one CFR, will greatly overshadow even the implications, little realised at the time, of commitment to building Concorde.

At first glance the benefits of fast breeder reactors seem overwhelmingly attractive. All present-day thermal nuclear reactors are fuelled primarily by fissile uranium-235. Even so, non-fissile uranium-238 is by far the dominant isotope in the fuel elements, and thermal reactors convert some of this to plutonium-239 which, like uranium-235, is fissile. But only a small amount is converted. This is where fast breeder reactors steal an advantage: they "breed" more fissile material in the form of plutonium-239 than is lost in the form of uranium-235. Obviously, this requires a major rethink of reactor design. A high density of fast, rather than slow, "thermal", neutrons is required, and the reactor core must be very compact. Consequently, the starting fuel must be a mixture of plutonium and uranium. The engineering difficulties are not insuperable; already prototypes have been built or are under construction, not only in the UK, but also in France, the Soviet Union, the US, and West Germany. Full-scale, commercial fast breeders would probably run at above the 1,000 MW mark, would be cooled by liquid sodium, and would, after the initial charge of fissile uranium and plutonium, never need specially enriched fuel, being able not only to run on depleted fuel from thermal reactors but also to feed back fissile plutonium to thermal reactors. All of which looks immensely attractive as a medium term prospect for nuclear power stations.

Except for one catch: all the plutonium which will be around. By the early 21st century, according to some scenarios, the world will be geared to a plutonium economy, with the developed nations satisfying their energy

requirements by using and circulating plutonium—a highly toxic metal quite easily convertible into nuclear weaponry. The commission accordingly devotes much of its attention to the problems that the plutonium economy throws up. It also, however, uses the opportunity of the report to review many other matters, such as radiological standards and waste management, which still needs serious attention regardless of any decision on the CFR.

The report first reviews what is known in radiobiology, with particular attention to plutonium. The present maximum permissible body burden is 0.6 mg, and the commission does not consider this standard to be seriously in error. In reaching this decision it had to consider the "hot particle theory", Gofman's analysis of the effects of smoking on lung clearance rates, and assertions that deaths from leukaemia among workers at the Windscale reprocessing plant in Cumbria were significantly high. For the first two, independent consultants were brought in, for the third the National Radiological Protection Board (NRPB) produced a paper, and, as a result, fears of any serious error in radiological standards were dismissed in every case. The consultants' reports have not been published nor even promised, and many will want to suspend judgment until they are openly available.

Next, the commission looks at how radiation standards are arrived at and enforced. The way that the International Commission on Radiological Protection reaches decisions is deemed satisfactory—the UK already fully accepts these standards. But there is some feeling that the NRPB, "a national point of authoritative reference in radiological protection", is not fulfilling a sufficiently central role, largely because it is fairly new while other bodies concerned with nuclear



power still have much in-house expertise. The commission recommends that consultation with the NRPB should become mandatory and that the NRPB should be statutorily responsible for endorsing basic standards. Further, the NRPB should provide the focal point for coordinating research into radioactivity in the environment, which is at present well done in the marine environment by the Fisheries Research Laboratory, but only patchily covered in the atmospheric and terrestrial environments. More work is also pointed towards the NRPB in the monitoring of radiation workers and of discharges.

If the comments on standards are relatively soothing, the pace of the report quickens a little when it comes to waste management. It is here, of course, that emotive issues—"leaving a radioactive time-bomb as a legacy for countless generations" and so on—could crop up. The commission asserts that "there should be no commitment to a large programme of nuclear fission power until it has been demonstrated beyond reasonable doubt that a method exists to ensure the safe containment of long-lived, highly radioactive waste for the indefinite future". It also makes it clear that the method does not yet exist. Lower levels of radioactivity are also considered and one or two comments made, particularly about the less than highest standards of housekeeping observed during the commission's 1974 visit to Windscale. But it is the high level wastes, mainly fission products, at present in tanks at Windscale which get most attention. Vitrification is seen as indispensable; development started in the late 1950s, but will not be commercially available until 1985 (there was a strange period of inactivity in the 1960s). Final disposal of the 15-tonne cylinders (a magnox reactor generates three tonnes a year in the UK) is the real problem.

The commission quickly rule out space rockets, subduction at ocean trenches, deep burial in Antarctica, and the time-honoured means of disposing of ships' waste—over the side. Burial is seen as essential, either on land or down specially drilled holes at sea. Surprisingly little research has been done in the UK on these options (maybe that is why they still seem attractive!), and the commission is critical both of the UKAEA and British Nuclear Fuels Limited for their complacency. It proposes that a Nuclear Waste Disposal Corporation be formed to get on with the job.

The two issues that get most headlines in connection with plans for widespread nuclear development are the possibility of serious reactor accidents and the risk of illicit activities. In both of these fields the commission acknowledges itself to be straying well outside

its brief, so its views can only really be those of a collection of very well informed laymen. Nonetheless, the chapters on these subjects, particularly on the latter, are important in that they provide a more total approach than is likely to appear in official discussions elsewhere.

Any assessment of the threat that terrorists could pose if a plutonium economy becomes established, or that security measures could pose to civil liberties is extremely difficult, and the commission rightly does little more than spell out some of the problems—it provides no cut and dried answers. On balance, the likely effects on civil liberties do not on the surface look serious in a generation used to all sorts of screening. But Sir Brian Flowers has already remarked that every wedge has its thin end, and even while the present arming of nuclear security guards must be seen as an eminently sensible move, the ultimate long-term implications need careful assessment. The terrorist threat looks more obviously bleaker. Would it be possible to site a fast-breeder in Northern Ireland? Is the rest of the UK immune from terrorist threats, bearing in mind that terrorist groups could be fed information by other states with nuclear capabilities? And the question is inevitably a world problem—if the UK opts for a plutonium economy it may significantly encourage other countries with less political stability to do likewise; even a terrorist attack in just one country would have worldwide repercussions. Still, the report carefully avoids judging these complex human questions, merely listing the potential dangers. But then, in a quite unexpected development, it proceeds to cast severe doubts on fast breeders.

The sudden switch comes in the chapter on energy strategy, and the argument runs like this. The Department of Energy (DEN) has made various energy demand forecasts for the next 50 years based on reasonable growth assumptions. In the most favoured current version electricity consumption more than trebles by 2025: 104 GW of nuclear electricity are needed by 2000 rising to 365 GW 25 years later. The commission observes that emphasis on electricity ensures that

- There will be a huge call for investment in the energy sector (3.4% of GNP, as opposed to 1.4% today),
- A large number of coastal sites must be developed by 2030—a requirement that it may not be possible to meet,
- A lot of the primary energy converted will be wasted in the form of low grade heat.

In a couple of breathtaking pages the commission becomes merchant banker and declares itself "very doubtful"

on the investment prospects, becomes the Fine Arts Commission and declares all these power stations a substantial adverse effect on amenity, and reverts to environmental commission to declare in three paragraphs that the heat might affect the climate. There thus appear "very considerable environmental objections to the high-nuclear, high-electric future". The feeling is rather as if a referee, after arbitrating over a goalless draw for 89 minutes, suddenly decides to boot the ball into the net to break the deadlock. Once the label "environmental objections" has been pinned on to one DEN scenario (although many other scenarios have been published—even down to a non-nuclear future) the rest of the report becomes coloured by a general optimism on alternative sources. Coal, of course, would bear the brunt of the load; and it is noticeable how the immense radiological protection lavished on nuclear workers is not matched by any comparable health concern for the miners who will have to dig the coal up.

One feels that with one hasty glance at 2025 the commission has made its mind up on the delicate issue of fast-breeder reactors—they should be introduced "only if demonstrably essential"; in the meantime money should be directed towards other technologies, notably fusion (though it is not clear that fusion research would benefit from a diversion of even a little of the £2,000 million that might eventually go into CFR-1), and international collaboration should be sought out for fast breeder development.

So where does this leave Mr Benn? The nuclear industry claims that it needs early decisions; the commission urges delay. Even though some of the commission's otherwise excellent reasoning is marred by sloppy thinking, the issues raised must give Mr Benn pause for thought. The question that undoubtedly is central in his mind is whether the technological momentum that will be built up by commitment to CFR-1 is stoppable if the warning bells begin to ring. Unless he can devise ways of ensuring this, and until the nuclear industry can demonstrate its understanding of the need for aborting mechanisms, he would do well to adopt a delaying strategy. The middle course steered by the commission makes one thing outstandingly clear: there is need for more discussion and a greater appraisal of the possibilities. Is an early decision on CFR-1 really as essential as the reactor itself may ultimately prove? The extra couple of months already gained since the report's publication (the DEN last week announced that the decision planned for October will now wait until at least the end of the year) must be seen to be more than just a token delay. □

Soviet dissidents (1)

He who would dissident be

Why do so many Soviet scientists become dissidents and refusniks? Vera Rich considers the question

ONE of the paradoxes of contemporary Soviet 'opposition', whether it comes from dissidents proper, who campaign for improvement of the system from within, or from the Jewish refusniks, whose only wish regarding the Soviet Union is to leave it for Israel, is the presence of large numbers of those who might be expected to benefit most from the *status quo*—scientists. In a culture which bases its whole economic policy on the "implementation of the scientific technological revolution" and which, with its total state control of all resources, can allot virtually unlimited funds to a favoured research project, the position of a scientist, one might feel, is surely such as to ensure all the fringe benefits which Soviet society affords its elite—access to privileged shops, better housing, Black Sea holidays, and the like. Yet, by the kind of paradox which characterises so much of Russian history, it is partly this privileged position that has led to the development of dissidence among scientists.

The story begins back in the late 1940s when Stalin, having 'abolished' Mendelian genetics throughout the Soviet Union, and replaced it with the peculiar notions associated with the name of Lysenko, turned his attention to modern physics. Since the basic tenets of relativity theory and quantum mechanics seemed, in the opinion of certain favoured theoreticians, contrary to Marxism-Leninism, Stalin aimed to confine physics research to what was congruous with Newtonian mechanics. But a small group of physicists, notably Kurchatov, Kapitsa and Vavilov, managed to convince him that without modern physics he could expect neither nuclear power, nor, and this was the clinching argument, a nuclear arsenal. Modern physics was accordingly permitted to continue, although, until Stalin's death, textbooks were liable to contain face-saving clauses to the effect, for example, that the second law of thermodynamics was "a local phenomenon in this part of the universe".

Having protected their own discipline from destruction, it was perhaps natural that physicists should come to the aid of their less-fortunate scientific brethren, the harassed geneticists, in their fight to re-establish genetics as a valid field of research. Lev Tumerman, a physicist working on the luminescence of organic molecules, was instrumental in arranging the first

seminar on chromosomes, held in the Lebedev Physics Institute during the late 1950s, while in 1959, Timofeev-Resovskii gave his first genetics lecture in Kapitsa's Institute. And in the great debate of 1964, which finally crushed the attempted resurgence of Lysenkoism and re-established genetics as a branch of Soviet science (although it did not receive 'priority' funding for another 10 years), certain physicists, notably Andrei Sakharov, played a significant role.

From the defence of a single scientific discipline to a deep involvement with human rights as a whole is, however, a great step to take. But a number of factors in Soviet society established a climate for dissent. The phenomenon of *samizdat*—'do-it-yourself' publishing and distribution, to sidestep official censorship—reaches far back into the 19th and 18th centuries, and it received a new impetus with the mushrooming availability of typewriters and, on occasion, photocopiers. The education reforms of 1958 which, in effect, made Russian virtually the only language of higher education and academic life, produced great discontent among the non-Russian republics of the Union, notably Ukraine and the Baltic States. The highly anti-Israeli attitude of the Soviet media at the time of the Six-Day War for the first time impressed upon Jewish intellectuals an awareness that Israel was not merely a paper creation (on the lines of the Soviet 'Jewish homeland' of Birobidjan). Israel at last came to be seen as a viable alternative society where Soviet Jews might live and work unhampered by the many disabilities which (*de facto*, and in contradiction to the Soviet constitution) are still the lot of those with "Jewish nationality" stamped in their internal Soviet passport.

But *samizdat* was, originally, the province only of the more daring creative writers, while the problems of minority nationalities had always been endemic to the multinational Russian empire. And except in a few cases, such as the promising Ukrainian school of cybernetics, little was lost to Soviet research when the language of instruction and publication was officially changed—a scientist, after all, must expect to read and, on occasion, to publish, papers in languages other than his own. Why then this great participation by the scientists in the human rights movement and dissidence?



Kronid Lyubarsky, 5-year sentence

Andrei Sakharov, the acknowledged leader of scientific dissidence, has given his own account. Though he originally viewed nuclear balance as an important factor of world peace, he subsequently began, as early as 1958, to agitate for the cessation of nuclear testing. The reason for the switch was clear. He became convinced that no further benefits could be gained from such tests, believing that the sole outcome would be nothing more than an increase in the natural level of genetic hazard. The fundamental change of attitude undergone by Sakharov by the early 1960s led to his concern about the 'criminal nature' not only of the tests but of the whole concept of nuclear weaponry. Beset with a sense of helplessness he devoted an ever-increasing amount of time first to *samizdat* writings (the 1968 monograph *Thoughts on Progress, Coexistence, and Intellectual Freedom*, and the *Second Manifesto* of 1970), and later to appeals on behalf of fellow dissidents threatened or imprisoned for their views and activities.

There is no doubt that Sakharov's personal charisma became a major factor in drawing together the little group who formed his illicit 'human rights' movement, and its subsequent off-shoots—the Moscow branch of Amnesty International and the 'Helsinki monitoring' group. Among Sakharov's associates are such leading lights as the physicists Valentin Turchin and Andrei Tverdokhlebov, the mathematician Valerii Chalidze, and the biologist Sergei Kovalev.

But what of the many others who did not at first have any personal contact with the great names of the dissident world? Some general trends may be distinguished. First, many students turn originally to science not so much from a personal preference for the subject as such, nor from hopes of a prestige job, but because science offers the hope of academic freedom, so

patently absent from the humanities which are dominated by the hard-and-fast requirements of Marxist-Leninist ideology. This expectation may remain unfulfilled—Soviet medical students are often disillusioned with psychiatry and psychology lectures based on Pavlovian behaviourism and a rigid definition of what constitutes 'normality'. Moreover, even in the absence of such specific disillusionment, the Soviet scientific establishment itself produces an atmosphere of frustration.

Travel to conferences abroad (or even, in some cases, within the Soviet Union) is frequently blocked by barriers of officialdom open only to the favoured few. Foreign publications cannot be obtained on subscription and are often inaccessible in libraries except to those with special security clearance.

The classic story of frustration recounted in *The Medvedev Papers* is by no means unique. The Soviet system makes no allowance for serendipity—a frequent complaint from young researchers is: "They expected me to order all the equipment and reagents before I started, but if I'd known exactly what I wanted, I wouldn't have needed to do the experiment"; or, "The official supply system is useless. You can only get on if your laboratory has a good 'fixer' who can arrange a swap with someone who has what you need." (This latter difficulty leads to deliberate over-ordering and the development of a system of polygonal bartering, involving several laboratories at a time.)

Such frustrations may eventually lead scientists to work more actively for human rights, even at the cost of a secure career. Vladimir Bukovskii, a biology student expelled from Moscow University for his dissident views, became the first prominent campaigner to focus world attention on the misuse of psychiatry by a regime aiming to equate dissidence with insanity. At the very least the frustration which so motivated Bukovskii may lead to a state of mind describable as "passive dissidence", creating a ready readership for available *samizdat* material. It should be remembered, of course, that even the temporary possession of *samizdat* material is a serious offence. Biologist Nina Strokata-Karavan'ska, for example, served a four year sentence for possession, and is still exiled from her native Ukraine. Similarly, Sergei Kovalev, another biologist, is serving a 7-year sentence in a strict regime camp. (Kovalev, a member of the Moscow Amnesty group was curiously charged with possession of the illegal *Chronicle of the Lithuanian Catholic Church*, although he is neither a Lithuanian, nor, so far as is known, a Catholic). Even greater penalties can be involved. Astrophysicist Kronid Lyubarskii, is not only serving a five year sentence—

moves are now afoot to deprive him of his academic degree.

For a Jewish scientist, however, discontent typically results in an application to emigrate to Israel. (As a convenient one-way ticket out of the Soviet Union, this opportunity is occasionally afforded to troublesome non-Jews, such as Leonid Plyushch, the Ukrainian mathematician.) But emigration is by no means an easy option. Successful applicants often indicate that the obstacles placed in their way actually intensify their Jewish consciousness, so that Israel becomes a positive permanent goal rather than merely the only available sanctuary. Once an application is placed, a whole chain of sanctions are imposed. Job dismissal is virtually automatic—with a consequent long-term loss of access to all professional publications and loss of contact with new specialist developments. (It was in an attempt to maintain at least some semblance of scientific life that Aleksandr Voronel founded the famous Sunday seminars). The authorities can throw up a whole battery of reasons for refusing exit visas. These include military service (often invoked in the case of over-age invalids, such as Mark Azbel, Voronel's successor as leader of the Sunday seminars); security restrictions (even in fields where the Soviet Union is not noticeably ahead of, or even abreast with, developments elsewhere); and the notorious 'education tax' (waived since the Nixon visit to Moscow, but never actually revoked) which required the payment of an extortionate sum, allegedly equal to the estimated cost of an applicant's university and post-graduate studies.

Reprisals may extend to relatives. Metallurgist Evgenii Reinberg was expelled from the Communist Party, and subsequently from his academic post, when his son applied for a visa. Endocrinologist Mikhail Shtern, now serving an 8-year sentence for "accepting bribes", was actually told that the accusation was made "in connection with your family's desire to emigrate". And, of course, dismissal from one's job involves the constant threat of arrest for 'parasitism', or being without visible means of support, a threat directed particularly often, it appears, at the members of Azbel's seminar.

Needless to say, a refusenik scientist shares the same disabilities as a dissident where international conferences are concerned. The recent absence of Benor Gurfel from the European meeting of the Econometrics Society in Helsinki, and of Academician Veniamin Levich from the Annual Meeting of the International Society of Electrochemical Scientists in Zurich, are cases in point.

'Active' dissidents and refuseniks con-

stitute, however, only a small part of the Soviet scientific community—indeed, a main campaigning point for refuseniks is that, since they are by no means irreplaceable, the Soviet Union can well afford to let them go. (Thus, such vocal and embarrassing dissidents, as Chalidze, Zhores Medvedev, and mathematician Aleksandr Esenin-Volpin, have been forced into exile.) So what of the bulk of the scientific community?

The overwhelming majority of scientists, it may be said, seem to find relief from discontent and frustration in 'inside emigration'—a complete absorption with work (although even that is a kind of dissent, since, according to *Pravda*, "a scientist's first duty is to be a patriot", with all that patriotism demands in voluntary community service and attendance at meetings and hortatory or celebratory rallies). The silent majority is, as everywhere, completely silent. Nevertheless, the scientific establishment still preserves some vestiges of independence from the State. The Academy of Sciences is the only remaining body in the Soviet Union to exercise the right of elections by secret ballot without official intervention (though it must be admitted that its current President, Aleksandrov, was nominated by the Party, before being duly elected). In spite of periodic press campaigns against Academician Andrei Sakharov, and in spite of the refusenik status of Corresponding-Academician Levich, the Academy has not, so far, expelled either. A two-thirds majority is needed for expulsion, and the lack of action so far indicates a degree of uncertainty as to how far dissidence, or fellow-feeling for dissidents, might spread.

In this situation of frustration, it is small wonder that so many appeals, whether from dissidents or refuseniks, call upon the world scientific community to take some action on behalf of harassed Soviet colleagues. The appeals are wide-ranging. Scientists in the non-communist world may be urged to plead for clemency for victimised Soviet scientists on trial. They may be urged to press for reviews of particularly harsh verdicts against those already convicted. Or they may simply be asked to request visas for specialists wishing to attend international conferences. A recent statement from the US Committee of Concerned Scientists stressed that these actions are more than gestures of common humanity towards distressed colleagues. When a scientist is for any reason prevented from working, the research he might have done is "irrevocably lost to science"; the fate of dissident and refusenik scientists affects not only Soviet science, but also the whole worldwide scientific community. □

Soviet dissidents (2)

Keeping the flame alight

Robert Adelstein participated in the Moscow Seminar earlier this year. Here he describes his experience

"THE West is serving as a witness to our scientific death. This is the whole point of the Soviet policy, to doom us forever as scientists". The speaker was Mark Azbel, head of the best known Moscow Seminar of dissident scientists. The words were the emotional highpoint of an impassioned closing speech delivered earlier this year during an extraordinary session of the seminar. For five days Hershel Markovitz, a Professor of mechanics and polymer science, and I, a biochemist, shared in the science, thoughts and experiences of the seminar members.

We had arrived in Moscow six days earlier not only to participate in the Moscow Seminar, but more important, to express the continuing interest of western scientists in their less fortunate colleagues. Any prior doubts we had about the impact of a visit by two relatively unknown scientists were quickly dispelled by the overwhelming reception we received. In honour of our arrival it was decided to hold a 'symposium' during which we would deliver three papers each and the Russian scientists would contribute ten of their own. We would meet every day for 3-4 hours of formal science followed by informal discussions about the status and problems of dissidents.

The Moscow Seminar on Collective Phenomenon, as it is officially known, convenes every Sunday at 12 noon in the sparsely furnished apartment of Mark Azbel. (It is one of six different seminars of dissident scientists convening in Moscow at present.) It comprises approximately 30 scientists drawn from various disciplines: physics, mathematics, cybernetics, electrochemistry, biophysics and molecular biology. But despite their differences in training the scientists all share one common attribute—they have applied for an exit visa to Israel and the visa has been refused. They are *refusniks*.

With very few exceptions *refusniks* are fired from their jobs and forced to eke out a living either by tutoring privately or by securing menial, non-scientific work. They are denied access not only to laboratories, but also to libraries, where their names are removed from published works, and where the books they have written are removed from the shelves. Reference by Soviet scientists to their published works is forbidden. They are denied official permission to publish scientific

papers in the Soviet Union or to mail them abroad to foreign journals (though papers can, of course, be smuggled out). Incoming mail, particularly from abroad, is interrupted. Telephones are often disconnected. Attendance at scientific meetings at home or abroad is forbidden. And *refusniks* are often ostracised by former colleagues and harassed by the KGB. The price of applying for an exit visa is high enough to discourage many would be emigrants.

Yet occasionally, in a pattern that defies discernment, a *refusnik* is released. My first visit to the Moscow Seminar had been in August 1972, the year it was first organised by 15 scientists because of "an urgent fear that we would lose our scientific standing, together with the loss of our employment". The words were spoken by Alexander Voronel, at whose apartment the seminar was convened. Three years after applying for his visa, Voronel was permitted to emigrate and is now a Professor of Physics at Tel Aviv University in Israel.

I remember sitting in Voronel's apartment with Benjamin Levich—a *refusnik*, a corresponding member of the Soviet Academy of Science, and a world-renowned electrochemist—together with his two sons Alexi and Yevgeny. His sons now live in Israel—though Yevgeny, a physicist, was incarcerated for a year in an Arctic Circle prison camp before being granted a visa. But their father and mother still await visas, despite Soviet promises to release them in October 1975.

My latest visit, in late winter, started on a Friday in a Moscow still blanketed with snow. My arrival with Hershel Markowitz, earlier in the day, was uneventful except for an extremely thorough search of our luggage at customs. Interestingly, the only reading matter which aroused enough curiosity to require further perusal by a higher official was not a month's supply of *Nature* and *Science* along with assorted scientific texts, but a Moscow guide book published in 1974 in the USA. It was our sole encounter with Soviet officialdom.

Later that night I journeyed by subway to the apartment of Irene and Victor Brailowsky—she a mathematician and he a cyberneticist—both of whom are members of the seminar. Arrangements were made to notify the seminar of our arrival (no easy task



Victor Brailowsky, harassed and threatened

since telephone communication was not possible) and to meet on Sunday for the trip to Azbel's apartment, which is located on another side of the sprawling city.

Mark Azbel greeted us enthusiastically on our arrival at his apartment. The balding, red-haired leader of the group looks considerably older than his 43 years. His whole manner reflects warmth and a certain dynamic intensity which seems to inspire the group whether discussing science or politics. After our introduction to the group—most in their 30s and 40s and much younger than I had imagined, we were joined by Benjamin Levich. A schedule for the 'symposium' was quickly drawn up, it being agreed to meet in Azbel's apartment every evening at six o'clock with the exception of Wednesday, when we would meet at Levich's. This would allow us to meet with some physical chemists who only attended Levich's Wednesday seminar, and with Alexander Lerner, like Brailowsky a *refusnik* cyberneticist. In one of the best examples of how international co-operation among scientists can force the Soviets to alter their policy, Lerner was permitted to attend an international meeting in Tbilisi, USSR, in August 1975, after western scientists threatened to boycott the conference.

The seminar on Sunday was brought to order by Azbel, raising his voice in a manner that brought to mind Moses at the Red Sea: "Yehudim Sheket"—literally, "Jews be quiet" (I have the impression that those are the only two Hebrew words he knows). Together, we covered a wide range of topics. Hershel Markowitz dealt with polymer rheology as well as with linear and non-linear viscoelasticity. I dealt with contractile proteins in muscle and non-muscle cells. Azbel discussed the decoding of DNA; Levich, charge transfer reactions in

solution; Victor Brailowsky, an algorithmic approach to diagnostic and prognostic medicine; Felix Lerner, electromicroscopy of muscle phosphorylase; and Edward Trifinov some biological consequences of clustering of pyrimidine photodimers in DNA. All of the lectures were presented in English, and translated into Russian for five or six seminar members who did not understand.

Trifinov, a 39-year-old survivor of the World War II blockade of Leningrad, whose father died in a Stalin camp, teaches his own course in molecular biology attended by the children of dissident scientists, among others. His first lecture coincided with the third day of the symposium, and he was terribly excited when I presented him with my last minute purchase, a new edition of Watson's *Molecular Biology of the Gene*.

At the conclusion of our first lecture, Azbel presented Hershel Markowitz and me with commemorative pins labelled with the initials MSCP—Moscow Seminar on Collective Phenomenon. The pins, awarded to all seminar lecturers, contain two lines symbolising uncoiled DNA and a balance—which Azbel pointed out as the "scales of justice".

Each evening when the scientific seminar was concluded, we gathered around a table for a snack, usually consisting of bread, cheese and fish, and the informal seminar commenced. Next to talking science, we spent most of our time discussing the plight of the dissidents, particularly why they had applied to leave, and what scientists in other countries could do to help. The two most frequently cited reasons for leaving were antisemitism and the corruption of scientific research by politics.

According to the members of the seminar, anti-semitism has become a sanctioned policy of the Soviet Government, manifested in the systematic exclusion of Jews from the better universities such as Moscow State. Moreover, Jews are being denied access to many professions so effectively that Alexander Lerner, who before his dismissal occupied an extremely important position, told me he applied to leave because "there is no future here for my children".

As scientists, Jews find the situation in the Soviet Union intolerable because scientific research is so enmeshed in politics. Promotion, or even survival in one position, requires constant political clearance, which in turn requires scientists to advocate political positions and sign political statements that they completely disagree with or know to be patently false.

So how can other scientists help? The seminar members stressed several possibilities. First, continued pressure must be maintained on the Soviet authorities

to allow free emigration; second western scientists should refuse to deal with Soviet scientists known to be instrumental in having dissident colleagues fired (a partial list of names is available); third, scientists, particularly members of official delegations, should visit the seminar and other dissident scientists (such visits, in the opinion of the seminar members, are instrumental in protecting dissident scientists from harassment by the KGB).

After meeting and eating with members of the Moscow Seminar for five days, Hershel Markowitz and I came away with the impression that they are indeed dying from a professional point of view. This isolation, as pointed out by Benjamin Levich, is particularly devastating for the younger scientists, whose careers and development demand constant work and access to recent publications. (Some of the work presented to us at the seminar was carried in 1971.) But despite their dire situation, they remain committed to their cause, and hopeful that help will come from scientific colleagues in other countries.

At an impromptu banquet held on the night before our departure, Hershel Markowitz and I augmented the usual diet with a chocolate cake, the lucky result of joining one of the many lines that suddenly form on Moscow sidewalks. The 'party' was tempered for me by two things. The first was a feeling of unreality brought on by the clash between a strong sense of identification with my dissident friends on Thursday night and the knowledge that on Friday afternoon I would be able to stop by my own laboratory to check on the latest results.

The second was Azbel's closing address. Besides warning of the potential scientific demise of dissidents, he pointed out that "although most Russian scientists are resigned to restrictions on their scientific freedom, for the dissident scientists this situation is intolerable. The dissidents would rather trade their prestige and, if necessary, their careers, for freedom. In this sense all scientists who apply to leave the Soviet Union must be understood as fighters for the freedom of conscience".

● Six months after my departure from Moscow little has happened to alter the status of the seminar members. Benjamin Fain, one of the few seminar scientists still working during my visit, has been fired. Several other members, including Azbel, Levich and Viktor Brailowsky have been harassed and threatened by the KGB, in an effort to get the seminar to disband. But still the meetings continue, soliciting visits from western scientists, among them the members of official delegations.

One month ago I received a letter smuggled out of the Soviet Union from Viktor Brailowsky. He and his two children have been granted permission to leave, but as often occurs there is a catch. Irene, his wife, is still refused permission—and the family will not leave without her. In his letter he appealed for scientists to send "letters, telegrams and telephone calls (the last are helpful) to R. V. Khochlov, Rector of Moscow State University", asking that she be permitted to emigrate together with her family.

Enclosed with Brailowsky's letter was a letter from another refusenik, German A. Shapiro, an endocrinologist and a former Assistant Professor at the Institute of Biophysics in Moscow. Discharged after applying for a visa in 1972, he now works as an ambulance attendant. The official reason for denying Shapiro permission to emigrate is that he had treated "patients who might know state secrets".

Two incidents illustrate that what at times appears to be a ludicrous situation, actually involves life and death. Tanya Levich has recently suffered a heart attack. Three years ago when the Soviet Government sent her son, Yevgeny, to a prison camp in the Arctic Circle, one of the reasons given for not letting Benjamin Levich emigrate was that it would be cruel to break up the family (since Yevgeny was obviously not free to leave the country). Now, having allowed Yevgeny and his brother out, the Soviets seem intent on making sure that Benjamin and Tanya never see their sons (and grandchildren) again.

The second incident involves another letter delivered by 'special courier'. While I was in Moscow, Alexander Lerner, knowing I had training in cardiology, showed me some electrocardiograms taken on Yefim Davidovitch. Davidovitch was a much decorated World War II hero who chose to sacrifice his career in an effort to emigrate to Israel. He was, however, quite ill, and clearly had heart disease. His friends, including Lerner, were concerned that he might not survive the difficult life imposed on refuseniks (his phone had been disconnected and he had been refused treatment by certain doctors and hospitals). After inspecting the cardiogram, I suggested that letters to some high Soviet health officials might be helpful in gaining his release on medical and humane grounds. I asked that a copy of his medical record be forwarded to my laboratory so that I and others could write on my return. Approximately two months after I returned, a detailed report on his medical history and physical examination arrived. Unfortunately, Yefim Davidovitch had died two weeks earlier. □

Soviet dissidents (3)

View from the Promised Land

Nechemia Meyers assesses the opportunities for Soviet scientists arriving in Israel

"AMONG the Russian immigrants are scientists on a par with the best in the US, and their coming to Israel gives this country a unique opportunity to develop many new research areas". So says Professor Alexandr Voronel, a Soviet expert in solid state physics formerly associated with the Institute of Physics at Chernogolevka, near Moscow, who joined the staff of Tel Aviv University. Voronel and his colleagues fear that this opportunity may be missed for lack of a coherent science policy in general and a plan for the absorption of these scientists in particular.

Exactly how many immigrant scientists have come here from the Soviet Union is impossible to determine, particularly because the term "scientist" covers a broader category of people in the East than it does in the West. Absorption authorities speak of 800 scientists and 400 graduate students (among the 100,000 Soviet immigrants who have arrived since 1971). Voronel thinks there are between 1,000 and 1,500, making up perhaps 15% of Israel's scientific community (as compared to about 25% of her doctors).

Those who have found employment in their professions work for the most part in universities and research centres. However, many hold temporary positions financed not by regular budgets but by special two- and three-year allocations from the Absorption Ministry and the Jewish Agency. When these allocations run out, the institutions where they are now working, which are so short of funds that they have had to fire some members of their academic staffs, will find it difficult to offer the immigrants permanent posts. In any case, future immigrants will find the universities closed to them.

This being the case, efforts are being made to channel immigrant scientists into industry. For example, the Centre for Absorption in Science announced that it was now sending 40% of its applicants to industrial firms, as compared to 10% 2½ years ago. However, as things stand, the absorptive capacity of Israel industry is also limited unless structural changes take place. For their part, the Russian immigrant scientists, or at least some of them, feel that Israel should pay heed not so much to the

American experience, as some have suggested, but to the Russian one.

"When I was in the Soviet Union," Professor Voronel stated earlier this year, "I was surprised that the Americans with all their resources and techniques had no scientific advantage over the Russians in many fields. Now that I am in Israel—that is to say within the framework of Western science—I wonder why the Russians are not doing even better, because from my vantage point I can see great advantages to the organized and planned system that exists in the USSR." Voronel says that with rare exceptions, such as the Manhattan Project or the attempt to land a man on the Moon, US research is based on individual initiative and achievements. In contrast, the USSR creates scientific teams geared to achieving specific national goals.

Voronel and his colleagues fully appreciate the freedom offered to them in Israel, which they would not forego in any circumstances; but they nevertheless believe that Israel requires a less "American approach" to science policy. "The US is big enough," Voronel declares, "to achieve success, without overall planning, in a great many fields. Israel, in contrast, will achieve very little without concentrating its efforts on a few well-defined projects, and this is where immigrant scientists from the Soviet Union can be of great value. They know how to organise and apply research though, of course, they must be better acquainted with the requirements of the Israeli economy before they are in a position to make concrete suggestions."

The Diamant brothers, Lev and Emanuel, are also drawing on the experience of Russian science in their attempt to create Arshach, a science-based community in the Galilee. They hope it will eventually expand into a kind of Academ Gorodok, the large Siberian research centre near Novosibirsk where Lev Diamant studied and worked in plasma physics before coming here. In spite of the fact that both brothers found positions in Israel soon after their arrival—Emanuel Diamant as an engineer at Tel Aviv University and Dr Lev Diamant as a physicist at the Weizmann Institute—they wanted to do more than just earn a living. They wanted to lay the foundations for the absorption of hundreds or



Alexandr Voronel, vantage point

even thousands of fellow scientists from the Soviet Union in a self-supporting scientific community to include both research and development and production facilities.

Their little settlement, perched on the Galilean hills not far from the spot where Christ gave his Sermon on the Mount, is no Academ Gorodok as yet. In fact, a lack of housing limits it to a few families and a modest research and development programme, most of it based on sub-contracts from established research centres in such fields as solar energy, custom-made electronics, electro-optics and lasers. Lev Diamant, who turned down US job offers, is not worried that the settlement is growing very slowly or that it is some distance from existing scientific and industrial complexes where computers, libraries and other important facilities are to be found. "In the US many scientists travel 60 miles, for example between Burlington and MIT, without thinking about it; why then," he asks, "should we Israelis make such a fuss about travelling 90 kilometers between Arshach and Haifa?"

The settlement has already established itself as an intellectual centre for immigrant scientists from the Soviet Union. The famous scientific seminars held in Moscow by Jewish scientists who had been dismissed from their posts after applying for an exit visa have been recreated at the village. They are headed by Professor Voronel, who was himself a key participant in the Moscow seminars before finally being permitted to leave for Israel last year.

Yet the purpose of these seminars, is different. "In Moscow," Voronel explains, "we were forced to concentrate on theoretical subjects because the authorities had taken away our laboratories and our instruments. Here we are discussing applied research, which we are trying, so far without too much success, to develop in Israel."

Soviet dissidents (4)

Trying to keep in touch

Yevgeny Levich describes the complex procedure involved in obtaining permits for travel abroad

WESTERN scientists welcomed the development of scientific contact with the USSR which came with détente. But enormous obstacles remain. A Soviet scientist intending to go overseas to take part in a conference or in some scientific exchange programme has to go through a complicated bureaucratic procedure, like any other Soviet citizen going abroad.

The first stage consists in obtaining a character reference (*kharakteristika*) which must be confirmed by the administration of his place of employment, by the local party organisation and his trade-union branch. The reference deals, among other things, with such matters as the applicant's participation in public activities, his moral image and his relations with the all grades of staff at his place of employment. Accompanying the reference is a detailed questionnaire covering the applicant's nationality, his party membership, details about all his former places of work and so on. The applicant is also asked to supply detailed information about his close relatives (including divorced spouse). When he has obtained the reference he is called for an interview to the Regional Committee of the Communist Party, where he is given the instructions which complete the open part of the procedure.

The main mechanism of issuing permits for foreign travel remains hidden from the eyes of the applicants. The outsized apparatus of the foreign Section of the Academy of Sciences of the USSR (General Korneev of the KGB has been head of this section for many years) or the ministries in charge of the various scientific research institutes are the bodies which deal with "travel matters". The central KGB has the last word in making decisions, which are communicated to the prospective traveller, as a rule, a day or two before his intended date of departure. There have been cases when a travel permit was taken away when the person was boarding the aeroplane.

In practice, the administration of the local institute (and especially its "first section"—the secret section which exists in every scientific institution and is connected with the KGB) plays an important part in making the decision. The deputy director of the institution, who is normally the head of the "first section", is also responsible for all international contacts. The information about the applicant which is passed on

through the closed channels has much more weight than his official reference. After the sanction of the Foreign Section has been received and confirmed by the KGB the "travel case" is transferred to the "Commission on Travel", headed for many years by the Politbureau's chief ideologue Mikhail Suslov.

The considerable efforts which are made to obtain permission to go abroad reflect the attraction that travel has for the Soviet scientist. Purely scientific considerations play the significant part here, of course, especially considering the almost complete isolation of the Soviet scientists in the recent past; but such academic interests are reinforced by financial attractions and considerations of prestige. Foreign travel is graded, like the Soviet elite's other privileges, according to value. Short trips to scientific conferences for which the scientist himself must pay are thought to be least attractive. It is much more difficult to be included in an official delegation going abroad at the state's expense. Then there is the rare possibility of going abroad for a long period of time, for example, within the framework of scientific-exchange programmes. Additional grading differentiates between travelling to the countries of Eastern Europe (not always easily obtainable) and travelling to the West.

The possibility of receiving a permit to travel abroad, and the efforts which must be undertaken to obtain it, are determined by a vague but identifiable correlation between the prestige value of the trip and the applicant's personal qualities. Being a party member, holding an official academic position, having personal connections in the administration and personal initiative are all to the applicant's credit; having relatives abroad, an unsuitable nationality, and a lack of interest in the party are not. Negative characteristics are usually critical and they automatically block off the entrance to some—if not all—ladders of privilege. On the other hand, some persons can arise to a level of the administration and party where they can by-pass the usual procedures and plan their trips more freely.

Moving up on the ladder of privileges and, in particular, gaining access to overseas contacts presents additional demands, the violation of which could mean the immediate loss of all that had been achieved. Scientific contacts between Soviet scientists and the West



Sakharov, attacked

are subject to fairly strict control. Two examples:

- Every scientist must present a detailed report, including not only details of scientific and industrial significance, but also characteristics of all the Western scientists whom he met, to the Overseas Section of the Academy of Sciences.

- Each scientist meeting with overseas colleagues at his home or at his institute must receive permission for such a meeting from the "first section" of his work-place. He must also present a detailed report about the meeting to the "first section" afterwards.

Evasion of these obligations automatically stops access to foreign contacts. On the other hand, some Soviet scientists deliberately overestimate the importance of their overseas contacts and their knowledge of scientific and industrial works in the West in order to increase their chances of a permit for their next trip.

Strict control applies not only to personal contacts, but also to scientific publications: all materials sent abroad, including letters to colleagues, must go through the special commission in every institute, which include representatives of the "first section". After being checked by the commission the materials must receive the sanction of the censorship office (the *Glavlit*). A particular form of control has appeared with the inclusion of Soviet representatives on the editorial boards of some international magazines, providing an opportunity to prevent publication of articles in foreign magazines.

Those who can travel abroad because of their position naturally enjoy significant advantages in the struggle for power which goes on in the upper layers of the Soviet academic community. But whereas scientific life inside the USSR is controlled to a significant extent by the representatives of the Soviet scientific elite, in matters connected with foreign contacts the

critical influence is concentrated in the hands of the party apparatus and the KGB. The selectively chosen representatives of the scientific elite have, however, merged with the administrative and party elite: those who have signed letters attacking Sakharov, those who have dismissed Jewish scientists wanting to emigrate from the USSR, and later signed false secrecy certificates of these scientists, are themselves scientists. Their actions are dictated not by their scientific but by their administrative interests, by their proximity with the party oligarchy and their desire to use this in their struggle for rank and power. They are the ones who appear most often at international conferences; they are the ones who gain most from the "scientific détente". The strengthening of scientific contacts

with the West, as long as they develop in a way suitable for the Soviet authorities, increases the influence of the most rigid elements of the Soviet establishment and provides them with additional levers to influence scientific life in the Soviet Union.

Soviet citizens differ from one another in everything, just as citizens of any Western country do. In accordance with the spirit of the "third basket" of the Helsinki Declaration, contacts ought to be developed on the individual level and not on the level of the delegations selected by the authorities. The organisation committees of international conferences should reserve the right to invite to conferences Soviet scientists who are prominent in their field, and should make their attendance a condition for the attendance of the

official Soviet delegation. The participation of Western scientists in any scientific exchange programmes must be conditional on the Western side being able to choose at least some of the members of the Soviet delegation and being able to have free contacts with any scientists in the USSR whom they would like to meet. Soviet scientists must also retain the right to send their articles to foreign magazines through members of the editorial board as well as through the Soviet regional editor.

Measures such as these would not cause a halt to scientific contact with the West. The needs of the state, and of the members of the elite, ensure that. And they represent something more than mere declarations of support or solidarity. □

COMECON

WITH growing industrialisation, fuel and power supplies are a matter of increasing importance to the Comecon block, particularly as the new policy of economic integration begins to take effect, and considerable reliance has come to be placed on oil, gas, and electricity delivered from the Soviet Union by pipeline and cable. But despite official Soviet statements that fuel and power export commitments would remain unaffected by the new drive for economies at home, it seems that there will after all be an export cutback. During a recent Miners' Day rally in northern Bohemia it became apparent that deliveries of Soviet oil to Czechoslovakia over the next five years would "fall short" of original estimates. Accordingly, the Czechoslovak Government has concluded that "we shall be unable to maintain the growth rate of past years".

There are other problems too. An article in the Slovak *Pravda* notes that in spite of the additional 4,000 MW or so of new generating capacity planned for the next five years (which should include two atomic reactors at Jaslovské Bohunice, providing some 35% of the total new capacity for Czechoslovakia), the generating system will still have "minimal reserves" and will be constantly stretched.

Nuclear power is a matter of pressing importance to Comecon, and, not surprisingly, there is considerable international cooperation. Joint research into the construction of the necessary equipment, which has resulted in the development of new types of generator, is carried out by Interatomenergo and Interatom-instrument. The latter organisation

is the only Comecon enterprise to have its own autonomous accounting system and operating capital of convertible currencies. Atomic power stations are being constructed (with Soviet aid and expertise) throughout the Comecon block, notably at Lake Żarnowiec in Poland, and Paks in Hungary. Ultimately, they should all



be connected with a 750 kV grid, the first line of which, from Ukraine to Hungary (with a later extension planned to Yugoslavia), should be commissioned in 1978. By 1990, nuclear power stations should meet one-quarter of the forecast electricity demand of the Comecon block.

In the meantime, to fill the energy gap, conventional fossil fuels are in ever increasing demand, and intensive work is being carried out both to modernise existing coal mines and to develop new ones. One such project, described recently by the Prague television service, bears a touch of inadvertent humour. A new open-cast coal mine, which will provide fuel for a power station in northern Bohemia, is, in line with the Socialist custom of naming important enterprises after

revolutionary heroes, to be called after Maksim Gor'kii. One of Gor'kii's most famous works is entitled *'From the Lower Depths'*.

● The drought which this year has affected all the European members of Comecon together with certain western parts of the Soviet Union (though not the Moscow region, where too much rather than too little rain has been the problem), has forced the authorities to place a new emphasis on the utilisation of water resources. Some research had, indeed, already begun with the mapping last winter and spring of Carpathian water-resources. That survey did not, however, extend to the eastern regions of the Carpathians (Ukrainian SSR), where over-zealous tree-felling is apparently resulting in erosion and consequent "destruction" of the water table. Precipitation in the Carpathians is of vital importance to the water supplies of Poland, Czechoslovakia, Romania, Hungary and Ukraine.

Several local water economy schemes are now being implemented. These include anti-flood measures on the Tisza, Mura and Raba in Hungary, a dam on the Cirocha in Slovakia, a special turbine-type aerator for the purification of polluted river water (developed at the Purification Equipment Institute in Sofia) and a reservoir on the Odra near Mietkowi in Poland. Although, like the Carpathian survey, many of these projects were already envisaged or under way before the drought became a pressing problem, the considerable media coverage they have received indicates that water supply has become an issue of grave importance.

IN BRIEF

Synthetic fuel block

A controversial plan to pump billions of dollars of federal funds into the commercial production of synthetic fuels in the United States was unexpectedly killed off in the House of Representatives last week. By the narrow margin of 193 votes to 192, the House blocked further consideration of a Bill to provide up to \$4,000 million for the construction of large-scale plants to produce oil from shale, and oil and gas from coal. The vote means that there is now no chance that Congress will pass the legislation before it breaks up for the November elections. The Bill was a major plank in the Ford Administration's long-term energy strategy, which calls for the production of large quantities of synthetic fuels by the mid-1990s to replace dwindling domestic supplies of oil and gas. It was defeated by an odd assortment of bed-fellows ranging from environmentalists fearing the pollution threat from conversion plants, to right-wingers opposing federal subsidies for industry.

Energy get-together?

Democratic Presidential candidate Jimmy Carter last week offered a plan to consolidate an assortment of government agencies in the United States into a single Department of Energy. Stating that "our country still has no energy policy" three years after the 1973 oil embargo, Carter said that the new department would include the Energy Research and Development Administration, the Federal Energy Administration, parts of the departments of Commerce and Interior, together with the Federal Power Commission. The plan would have a tough time getting through Congress if Carter is ever in a position to propose it formally, however. Former President Nixon twice suggested the setting up of a Cabinet-level department of energy and natural resources, but the reorganisation was never approved by Congress because it would have cut across the jurisdictions of many Congressional committees, which now roughly match those of existing departments and agencies.

Bangladesh council

The Government of Bangladesh has constituted a National Council for Science and Technology to plan the country's scientific and technological programme and activities. The high powered council is chaired by the President of Bangladesh, and one of the two vice-chairman is an eminent scientist who is also the Science Adviser to the President, Professor M. Innas Ali.

The council has 19 other members who are *ex-officio* chairmen of research councils, secretaries of some ministries, Vice-Chancellors of two technological universities and two reputable Bangladesh scientists or technologists nominated by the President. Professor Ali himself has a distinguished career in scientific research, education and administration. He holds a Master's degree in electric engineering from the University of New York, a doctorate in nuclear physics from the University of London; and is a former Vice-Chancellor of Chittagong University.

SECRECY, in science as in politics, is a delicate and difficult subject. There is much concern when the details of Cabinet deliberation are prematurely revealed, yet at the same time there is a strong campaign for the relaxation and rationalisation of the Official Secrets Act. It is difficult to strike a happy medium. The results of scientific research concerned with military problems or commercial activity may legitimately be kept secret for a time, but it often seems that publication of much of this work is delayed unnecessarily. Some results which might have a wide application never see the light of day, even when the defence of the country is no longer at risk, and when no benefits would immediately accrue to a company's commercial rivals.

The reputation of a scientist depends largely on the quality and quantity of his published work. If he is unable to publish, he is likely to remain unknown except among his immediate colleagues. It is therefore important to the individual, as well as to the scientific community, that as few restrictions as possible be placed upon the publication of the results of any kind of research. High salaries, or even the award of honours and decorations to otherwise unknown scientists may be poor recompense for the denial of proper recognition within the scientific community.

When the reorganisation of

government-supported research attributed to Lord Rothschild was being discussed, there was some concern that it might have a harmful effect on the dissemination of scientific know-

In confidence**KENNETH MELLANBY**

ledge. It was feared that the executive government departments, to whom so much of the research funds was to be transferred—so that they, as "customers", could place "contracts" with research councils or other laboratories—might be reluctant to allow the scientists who did the work to publish their results in the accepted scientific journals. These particular

fears appear to have been unfounded. Some senior ministry officials have suggested that since they paid for the research, they should have the right to decide what should and should not be published. But it is to the credit of Chief Scientists that they normally insist that those engaged in research should be free to publish as they wish. Indeed, in some cases contracts have included the proviso that results should be made available to the customer first, and there seem to have been few cases where final publication has been seriously delayed.

And yet the new system appears to have reduced substantially the amount of material being published by some of the laboratories now largely engaged in contract research. The reason would seem to lie in the nature of the contracts themselves. Few contractors seem to have followed the example of the Ministry of Agriculture, Fisheries and Food, whose contracts have usually been for very large sums, and have covered substantial fields of study, so as to allow room for manoeuvre and originality. Too many contracts have been small and circumscribed by limited objectives, presenting few opportunities for research workers to make significant advances. There has consequently been little worth publishing. And in that situation, it does not matter whether or not the workers are pledged to secrecy.

news and views

Complementary mispairs

from E. G. Richards

THE fidelity of DNA replication is a matter of crucial importance: too low, and life as we know it is impossible; too high and there is no evolution for us to ponder the matter. The observed frequency of mutations and replication errors must depend on several factors, including the nature of the error, the chemistry of the replication machinery and the fundamental chemical properties of the bases in DNA themselves.

An interesting theory has been propounded by Topal and Fresco (*Nature*, **263**, 285; 1976) to account quantitatively for the observed frequencies of a certain class of mutation—point mutations which entail the substitution of one base pair for another in the DNA.

Such point mutations can be of two sorts: transversions in which an A–T or G–C base pair is substituted for its opposite number, namely a G–C or A–T pair respectively; and transitions in which the pair is reversed between the two DNA strands so that an A–T pair becomes T–A or G–C becomes C–G.

As is often stated, the Watson–Crick base pairs, A–T and G–C, are each complementary. Among other things, this implies that both sorts of base pair are geometrically similar and can substitute for each other in the regular DNA helix. The crucial observation, based on model building, of Topal and Fresco is that there are other sorts of base pair which are geometrically similar to the normal Watson–Crick pairs. These they have termed complementary mispairs, and they are formed from bases which are thermodynamically rare forms such as the enol or imino forms or in the *syn* rather than the normal *anti*-configuration (obtained by rotating the base through 180° about its glycosidic bond).

In the replication process, each base in turn selects, with the aid of the enzymes involved, a complementary partner which is incorporated into the growing chain. If on rare occasions a base, instead of selecting its normal partner to form a normal Watson–Crick base pair, selected another partner to form a complementary mispair, an error or mutation would be born. Topal and Fresco find that all possible transversion and transition mutations could come about in this way.

Considerable credence is lent to this idea by the report that base oppositions corresponding to each of the required complementary mispairs have been demonstrated experimentally to take up intrahelical conformations in long double stranded polynucleotides dominated by normal Watson–Crick base pairs, whereas other base pairs which are not complementary and are not required take up extrahelical conformations instead.

So far one might suppose that the frequency with which a transversion or transition occurred would be determined mainly by the proportions with which the different nucleotides exist in their rarer tautomeric or isomeric forms. These proportions are determined by the thermodynamic properties of the bases and have in fact been measured. The results suggest that the enol or amino forms would occur at a frequency of 1 in 10^4 – 10^5 and the *syn* form at 1 in 10–20. These frequencies are however far higher than those at which point mutations are observed to occur—1 in 10^9 – 10^{12} base pairs replicated.

In circumventing this difficulty, Topal and Fresco note that there is good experimental evidence that replication takes place in two stages. In stage one, the replicative machinery holds the template base in position and

presents a hole into which only a complementary base may fit to be joined to the growing strand. At this stage a mispair may be formed with the frequencies noted above. In the second stage a checking mechanism is brought into play which ascertains if the newly formed pair has standard geometry. If not it is excised. Topal and Fresco propose that before the check is completed, thermodynamic equilibrium is re-established and the tautomers revert to the keto or amino form with a corresponding disruption of the geometry (because of stacking interactions it is likely that the *syn-anti* equilibrium is frozen). At frequencies determined by the same thermodynamic considerations, however, a small proportion of mispairs will remain mispaired and not be detected. Thus the proportion of mispairs that are both formed and survive the checking step will be approximately the square of the proportions mentioned above. Topal and Fresco have worked out in detail from their proposed mispairs and the relevant thermodynamic data the frequencies at which they would expect the various sorts of point mutation to occur. They find a remarkable agreement with data obtained from a study of the frequency of spontaneous mutations in the tryptophan synthetase A gene of *Escherichia coli*.

One interesting feature in the proposed base mispairing schemes is that transitions arise only from purine–pyrimidine mispairs and transversions only from purine–purine mispairs. It is therefore of interest and wholly consistent with Topal and Fresco's hypothesis that the base analogue 5-BrU has the specific effect of enhancing the frequency of transitions. 5-BrU also has a much greater tendency to enolise than its natural counterpart T. Similarly they can explain the specific effect of

2-AP in inducing trasversions. They go on to comment that chemical mutagens (many of which are carcinogenic) may act by disturbing the tautomeric or isomeric equilibria of bases with which they interact.

This hypothesis is certainly elegant but as the authors point out, further experiments are required to assure its validity. Be this as it may, if it is valid, similar effects may well occur in other situations in which complementary base pairing is invoked. In particular it may occur in transcription and in mRNA interactions with tRNA during translation on the ribosome. Such matters are the subject of a second paper by Topal and Fresco (*Nature*, **263**, 289; 1976).

Any discussion of codon-anticodon interactions in translation must be dominated by Crick's wobble hypothesis. Topal and Fresco devote part of their paper to a discussion of what base pairs could and could not occur in the third codon wobble position. One suggestion they come up with is that in the wobble pair A-I, the A residue on the codon may be in the

syn configuration rather than the *anti* as proposed by Crick. This would give a glycosidic bond separation close to the standard 10.9 Å instead of the longer value of 12.8 Å.

Whatever the precise nature of the wobble pairs may be, mispairing would lead to errors in amino acid incorporation. The *in vivo* rate of such errors is as high as 1 in 10^4 . This figure, Topal and Fresco surmise, results from mispairing in the transcription process as well as in all three positions in the codon-anticodon interaction. The calculation of the value expected on the basis of mispairing schemes and the tautomeric equilibrium constants is complicated by the degeneracy of the code, but ignoring this difficulty, Topal and Fresco arrive at a value of 1 in 4×10^4 for the overall error rate. Presumably there is no checking process analogous to that in replication. This may be because the errors can arise at two distinct stages—transcription and translation—at roughly the same frequency so that the economics of the cell would render it unprofitable to check errors at one stage only. □

climates to observe the shape of a single flagellum, even though its diameter is only 20 nm. The advantages of this over electron microscopy are that distortions and environmental changes induced by specimen preparation can be avoided, and phase transitions observed directly while the medium is changed. Such transitions include a change from left- to right-handed helices on reducing the pH.

Kamiya and Asakura describe how this change occurs in reconstituted *Salmonella* flagella. The change is rapid and reversible, and can be seen to start at one end of a flagellum and propagate down its length. As this happens, the transformed region appears to rotate rapidly round the remainder, which in their experiments was tethered to the slide. Similar effects can be induced by hydrodynamic forces, and Hotani's ciné film of this sort of motion has recently been gripping scientific audiences around the world. The experiments show clearly that the transformation is due only to a reorientation of the subunits, in a process akin to that postulated by C. R. Calladine (*Nature*, **255**, 121; 1975) to explain the different static waveforms that can occur.

Hotani's companion paper concentrates on the static appearance of flagella of mixed type, in which polymerisation is started with one monomer mixture and finished with another. As well as measuring accurately the parameters of the helices, Hotani measured the "block angle" between the axes of helices at the point of changeover. There seems to be no kink in the structure, but the transition from one form of packing to another causes the new helix to coil about a different axis. This is particularly marked when the handedness reverses; the block angle can then be as small as 94° . The most interesting feature of Hotani's results is that nearly all the data fit a simple equation relating the block angle to the pitch angles of the two helices concerned. This equation demands a condition which also underlies Calladine's prediction of helical polymorphs.

This prediction followed the observation that a flagellum is made up of longitudinal rows of subunits which generally make a small angle with the rod axis. The large scale helices adopted are consistent with some of these rows running always along the inside of the helix, while other rows (of slightly greater length per turn of helix) run always along the outside. Calladine showed how "short" rows could arise by rearrangement of the connections between subunits, and he argued on purely mechanical grounds that such rows should cluster on one side of the rod. A transition between polymorphs would then involve a

Structure of bacterial flagella

from Michael Spencer

BACTERIAL flagella fascinate biologists, molecular and otherwise, for a number of reasons. They are built up from identical subunits; they are helical (look what that did for Watson and Crick); and they constitute a largely unsolved problem in motility. It seemed a couple of years ago that the important question of whether they rotate bodily or merely execute helical waves had been settled in favour of the former idea; certain lines of indirect evidence convinced many scientists that there must be some kind of molecular motor, complete with bearings, at the base of each flagellum. However, direct evidence for the functioning of such a device has been lacking, and a minority remains sceptical. The case for the new doctrine was summarised in a persuasive article by Howard C. Berg entitled *How Bacteria Swim* (*Sci. Amer.*, **36**, August, 1975).

Meanwhile a number of workers have refrained from committing themselves firmly to any hypothesis, and have continued to publish new structural data. The most prolific of these has been Sho Asakura of Nagoya University, who has specialised in the

repolymerisation of flagella *in vitro*. By mixing seeds and monomers from various mutants, a number of flagellar forms can be produced which vary in the pitch, amplitude and handedness of the large-scale helix into which the basic rod structure is coiled. Others have used optical diffraction of micrographs to study the detailed packing of subunits (for example, Kondoh and Yanagida, *J. molec. Biol.*, **96**, 641; 1975). It is unfortunate that almost every worker in the field has invented a new terminology to describe the helices, and evocative but imprecise terms such as "curly" and "semi-coiled" have served to keep the non-specialist confused.

The two most recent publications in this field are by Hotani now at Kyoto University, and by Kamiya and Asakura (*J. molec. Biol.*, **106**, 149 and 165; 1976). Both papers describe the use of dark-field light microscopy, which has recently undergone quite a renaissance. Thirty years ago it was used by Pijper to observe flagellar bundles in motion, using sunlight on the roof of his South African laboratory. By using a high-intensity lamp it is now possible for workers in shadier

change in the number of "short" rows, but not in the side on which they clustered. Hotani's results are consistent with this argument.

What is intriguing is that the kind of structure described is also required by the basic "non-rotary" hypothesis for generation of helical waves, an idea with a lineage going back to 1883. On this hypothesis the packing pattern would be induced by some mechanism at the base to propagate round the rod, giving rise to apparent rotation. Although Hotani follows Kamiya and Asakura in eschewing speculation, their results taken together suggest that there could yet be some life in the old idea. □

The folded chain's last stand?

from Paul Calvert

In the past two years low angle neutron scattering studies on amorphous polymers have shown that the chain dimensions in both the liquid and glassy states are equal to the random coil dimensions seen in ideal solution. This scotched the idea that there are ordered regions in amorphous polymers but has left the puzzle of how to pack chains to high density yet retain the random coil (Kirste *et al.*, *Polymer*, **16**, 120, 1975; Cotton *et al.*, *Macromolecules*, **7**, 763, 1974; Wignall *et al.*, *Eur. Polymer J.*, **10**, 861, 1974).

Results of similar experiments on crystalline polymers are now being published and very surprisingly show that the chain is still in a random coil. This one fact contradicts much of our current understanding of polymer crystallisation.

The small angle neutron scattering experiment is similar to small angle X-ray scattering except that the contrast arises from the very different scattering powers of perdeuterated and protonated polymer rather than from density differences. Usually a few per cent of normal polymer is mixed into a matrix of wholly deuterated chains. The strong incoherent scattering from the protonated chains is measured as a function of angle and allows their radius of gyration and molecular weight to be determined. For amorphous polymers the radius of gyration was found to be equal to the ideal solution value. The apparatus necessary is a nuclear reactor with a thermal neutron beam and a diffractometer. Experiments have been done at Harwell but the beam intensity there is low and much more can be done on the purpose-built research reactors at Grenoble and Jülich

Was the early Solar System windswept?

from David W. Hughes

If the nebula that formed the Solar System had the same elemental composition as the Sun, the condensates, that is the planets, asteroids and comets which make up our present Solar System, represent only about 5% (by mass) of the original nebula. The remaining 95% has been lost in the intervening time between planetary formation and the present. Most modern cosmogonical theories use the solar wind to sweep the newborn Solar System free of this "left over" nebula which is made up mainly of volatile elements such as hydrogen and helium. This solar wind is a cloud of particles (mainly protons) which are streaming away from the Sun. At the present time the average cloud density near the Earth is 5 protons cm^{-3} and the particles stream past at a speed of about 450 km s^{-1} . In the T Tauri stage of the Sun's evolution this wind was blowing at about the same speed but was much denser, the Sun losing mass at a rate which could have been as high as 10^{19} g s^{-1} . This T Tauri stage lasted around 50 million years during which time the Sun's radius decreased from nearly that of the radius of Mercury's orbit to its present $7 \times 10^5 \text{ km}$ and the Sun lost a considerable percentage of its mass. The Sun was also a variable, surrounded by a thick, highly active chromosphere. Gravitational collapse was giving way to thermonuclear hydrogen burning as the main energy source.

M. J. Handbury and I. P. Williams (Queen Mary College, University of London) question the wind-sweeping

hypothesis in a recent paper in *The Observatory* (**96**, 140; 1976). Assuming that the solar gaseous nebula is in equilibrium about the Sun before the onset of the wind the authors then calculate the rate of momentum transfer between a continuous, spherically-symmetric wind and the nebula. Three forces are acting on the nebula, gravity, "centrifugal force" due to its rotation and the solar wind force due to its rotation and the solar wind force due to momentum transfer. As angular momentum is conserved a formula can be easily obtained for the new equilibrium distance between the nebula and the Sun. Handbury and Williams find that for all reasonable values of the parameters the nebula does not get pushed out to infinity by the T Tauri wind but simply increases in radius.

Now if energy transfer and not momentum transfer is the actual mechanism the total nebular energy could become positive and the nebula would disperse. But this would require the kinetic energy of the wind to be converted into thermal energy in the nebula and then back again into kinetic energy as the nebula moves away. Also loss due to radiation, evaporation or fast moving particles and other mechanisms has to be negligible.

So the authors conclude that the solar wind cannot be the broom which swept from the Solar System those elements that did not condense to form the planets and that cosmogonists must look for another process.

(see Schmatz *et al.*, *J. appl. Cryst.*, **7**, 96, 1974; for a general description of small angle neutron scattering).

The first experiments on crystalline material showed that the scattering was dominated by scattering from the matrix attributed to submicron voids. This effect could be eliminated by using deuterated polymer in protonated matrix rather than *vice versa* as the scattering lengths of the protons and carbons almost cancel. It then became clear that segregation was taking place so that the deuterated polymer was clustering in the crystals as the measured molecular weights were many times the true value (Schelten *et al.*, *Polymer*, **15**, 682, 1974; *Colloid and Polymer Sci.*, **252**, 749, 1974). This arises because deuterated polyethylene melts at about 6°C below the protonated polymer.

The next set of results to be pub-

lished were those of Sadler and Keller (*Polymer*, **17**, 37; 1976). They measured polymer grown single crystals in the range of scattering angle appropriate to the interchain spacing in the crystal rather than the chain as a whole. They showed that their results can be explained in terms of an adjacent re-entry model but could not really eliminate alternative models. These experiments may have been affected by clustering which could invalidate the results.

During this summer results on melt crystallised polymers have been reported at several conferences by Ballard, Wignall and Longman from ICI at Runcorn using the Jülich diffractometer (to be published in *Polymer*). They tried to eliminate clustering by using a branched chain protonated polyethylene in conjunction with a linear deuterated polymer so that the crystallisation temperatures

matched. They found that even with such matching they could not eliminate segregation except in samples crystallised by quenching. Neutron scattering measurements were made on these samples down to low enough angles to observe the radius of gyration of the whole chains. For quenched samples they found a value of ratio of radius of gyration to the square root of molecular weight $\langle S^2 \rangle_w^{1/2} / M_w^{1/2} = 0.46 \pm 0.05$, the same as for molten polymer and ideal (θ) solution. Over the molecular weight range from 40×10^3 – 400×10^3 it is not possible to tell whether there is a change in the ratio. At concentrations of deuterated polymer less than 10% the quenched samples gave correct molecular weight values showing that no clustering occurs. Slowly crystallised samples give a molecular weight value several times too high due to clustering.

The significance of these results is that they undermine the folded chain theory of crystallisation in which each polymer chain crystallises in a 'jumping jack' configuration, repeatedly traversing the crystal thickness of about 100 atoms (a stem) then folding back on itself. The chain will thus have the shape of a thin sheet. This model is supported by a large amount of indirect evidence on the morphology and properties of single crystals grown from solution and by the Lauritzen-Hoffman theory of crystal growth which correctly predicts the crystal growth rates from melt and solution on the basis of adjacent re-entry folding.

One direct experiment is that of Bank and Krimm (*J. Polymer Sci.*, **A-2**, **7**, 1785; 1969) who studied the infrared spectra of mixed perdeuterated and normal polyethylene and showed that even at low concentrations a deuterated stem is likely to be adjacent to other deuterated stems so that the chain must fold back on itself. The Sadler and Keller neutron experiments are equivalent to this. Mandelkern and coworkers (*Macromolecules*, **4**, 672; 1971; reply, *ibid*, **5**, 209; 1972) have raised the question of segregation in the Bank and Krimm experiments but could not really prove it. The new neutron experiments seem to have verified Mandelkern's idea and invalidated the infrared experiments. The main alternative model to adjacent re-entry folding is the 'telephone switchboard' or non-adjacent re-entry proposed by Flory but a few months ago the majority of polymer scientists accepted adjacent re-entry as the norm.

Ballard and coworkers discuss their results in terms of two models. The first, assuming the chain is in a random coil configuration, gives a good fit to both the radius of gyration and the shape of the scattering function. If adjacent re-entry folding is assumed, a fit can be obtained if the chain folds a

specific number of times then moves to a new crystal, but the constraints on the number and length of folds are such that it becomes difficult to explain the molecular weight dependence of the data. Thus the most satisfactory approach is to assume that the chain crystallises with no gross distortion of the random coil which exists in the melt, though local changes must occur. A search for other models which fit the data is being made.

These results only apply to quenched samples as the clustering disqualifies results on slowly cooled and solution crystallised samples. Defenders of chain folding can thus say that it will still apply to crystallisation nearer to equilibrium. This is unsatisfactory so long as there is no prediction as to where 'near equilibrium' ends. One major omission of theories to date is that there has been no comprehensive attempt to treat the dynamics of the chain as it attaches to the crystal. If crystallisation is slow the chain should have plenty of time to relax its shape

to a 'jumping jack' as it is attached but when the crystals are growing rapidly they may well just entrap chains with little relaxation from the random coil. At the moment there is no basis for saying where the boundary between these two regimes lies. Chain folding theories assume that the first case applies over most of the normal range, my personal opinion is that the second case may be the rule, possibly even in solution crystallisation.

In the next year we may expect more neutron results on other systems. Ideally a polymer may be found where the labelled and unlabelled chains do not segregate significantly during slow crystallisation but since the chain motions involved in segregation and chain folding must be related we may reach an impasse. The adjacent re-entry chain folding model has received a nasty blow. If it does not survive as the description of melt crystallisation much of our understanding of polymer structure and properties will have to be reassessed. □

Radioastronomy and cosmology

from M. S. Longair

The cosmological problem as it presents itself to the radio astronomer was the subject of IAU Symposium No 74, Radio Astronomy and Cosmology, which was held at the Cavendish Laboratory, Cambridge on August 16–20, 1976.

THE standard textbooks on cosmology describe how the density, deceleration and geometry of the Universe may be determined from observations of the most distant galaxies. The remarkable fact that even the brightest extragalactic radio sources are at very great distances means that in principle they can be used in all the classical cosmological tests. In practice radio sources tell us rather little directly about these properties of the Universe because the overall properties of the radio source population evolve rapidly with cosmological epoch. Radio sources are associated with the most violently active systems—the nuclei of massive galaxies and quasars—and in general terms the distribution of radio sources traces how this violent activity has changed with cosmic time. All the evidence suggests that the Universe went through a period of violent activity when it was about one-tenth of its present age. Thus the contribution of discrete radio sources to cosmological problems is directed much more towards physical

aspects of the evolution of the Universe than towards the geometrical questions of classical cosmology.

The emphasis of the symposium was on the ways in which observations of discrete radio sources provide information of cosmological relevance. In interpreting the data, attention was focused on standard evolutionary world models.

The overwhelming impression was of the wealth of high quality observational data which has become available in recent years at radio and optical wavelengths. The radio observations span a range of 10^4 in sensitivity and the associated optical objects span a range in redshifts of at least 0 to 3.53. The radio surveys have been directly responsible for the discovery of large numbers of very large redshift quasars.

All such cosmological studies are ultimately based on surveys of radio sources and very large samples of sources are now available at frequencies of 0.408, 1.4, 2.7 and 5 GHz. Perhaps the simplest of all cosmological tests at radio wavelengths is the counts of radio sources which are shown in Fig 1 in differential form. The number of sources ΔN in the flux density interval ΔS is shown normalised to an arbitrary Euclidean count $\Delta N_0 \propto S^{-5/2} \Delta S$. A major advance has been the great increase in the numbers of sources counted at flux densities $S \leq 1$ Jy at all frequencies. The be-

haviour of the counts at 0.408, 1.4 and 2.7 GHz is similar, showing three distinct regions: at high flux densities, $S > 3$ Jy, the observed numbers of sources increase relative to the Euclidean prediction with decreasing flux density; at intermediate flux densities, $0.1 < S < 1$ Jy, the numbers follow closely the Euclidean prediction; at the lowest flux densities, $S < 0.1$ Jy, the numbers of sources converge relative to the Euclidean prediction. It has been known for many years that this behaviour contradicts the result expected for a uniform spatial distribution of sources which is that the counts should be a monotonically decreasing function of decreasing flux density and is evidence that the properties of the radio source population have evolved with cosmological epoch. As the frequency of observation increases, the "maximum" in the normalised differential counts broadens appreciably due to the presence of a larger proportion of sources with flat radio spectra. At 5 GHz this broadening is so marked that the counts follow closely the Euclidean prediction over a wide range of flux densities. In view of the small difference in frequency between 2.7 and 5 GHz, such a difference is remarkable and suggests that a new class of radio source with flat radio spectra appear at flux densities $S < 0.1$ Jy. Several lines of evidence suggest that this new class may be weak radio galaxies.

In the past there have been suggestions that there are anisotropies in the distribution of sources over the sky. The large surveys of sources made by observers at Bologna, Cambridge, Molonglo, NRAO and Parkes have now been analysed using the powerful technique of power spectrum analysis and no evidence for anisotropies has been found. The Westerbork observers reported the results of many surveys made in the flux density range $S_{1.4} < 0.1$ Jy where the source counts converge and no evidence of anisotropies was found. A few claims of anisotropies were made—for example, differences in the total numbers of sources detected in different sections of the Molonglo and NRAO surveys which are significant at about the 1% level. These results must however be compared with the large body of data which reveals no significant evidence of anisotropy. A. S. Webster (Institute of Astronomy, Cambridge) pointed out that this provides the best evidence for isotropy of the distribution of discrete objects in the Universe on scales ~ 1 Gpc.

The angular diameter-redshift relation is an attractive cosmological test because all the standard world models predict a minimum in the angular size of a standard rod with increasing redshift. Since radio sources extend to

large redshifts, many observers have searched for such a minimum using the physical size of radio sources as a standard rod. R. C. Roeder (David Dunlap Observatory, Toronto) reminded observers that this result is only true if the matter distribution in the Universe is uniform; in completely inhomogeneous models, the minimum disappears. Observations of the relation for radio galaxies and quasars with redshifts up to 2.8 were reported by groups from Cambridge, NRAO and Westerbork. All that could be said at present was that the angular size distribution of quasars observed at large redshifts appears to agree with what is predicted from the known physical size distribution at small redshifts although the statistics are still small.

A slightly different result is found for the related test, the angular diameter-flux density relation. R. D. Ekers (Kapteyn Laboratory, Groningen) extended the earlier work of G. Swarup and his colleagues (Radio Astronomy Centre, Ootacamund). The median angular size of complete samples of radio sources decreases with decreasing flux density until the very lowest flux densities, $S \lesssim 0.1$ Jy, at which the median attains a roughly constant value. It is not yet known if this is a minimum or an asymptote. Calculations by V. Kapahi (Tata Institute, Bombay and Groningen) showed that even the decrease in the median angular size with decreasing flux density disagrees with the postulate that the physical sizes of radio sources are independent of redshift. His results suggest that the median physical size must be smaller at large redshifts, roughly by a factor $(1+z)^x$ where $x \approx 1$ to 1.5. This result does not necessarily contradict the direct observations of the angular diameter-redshift relation for quasars—Kapahi's result refers to all radio sources, the other to quasars alone.

Optical identification of radio sources has proved to be one of the most successful techniques for finding very distant galaxies and quasars. R. Fanti (Radio Astronomy Laboratory, Bologna) and C. Perola (Institute of Physics, Milan) showed that for weak radio galaxies there is a correlation between their radio and optical luminosities but for powerful radio galaxies, as has been known for many years, the dispersion in absolute optical magnitudes is small and is independent of radio luminosity. This means that the galaxies associated with powerful radio sources may be used in the optical redshift-magnitude relation. H. E. Smith (Physics Department, La Jolla) showed that in the past few years this approach has led to the discovery of eight radio galaxies with measured redshifts in the range 0.467 to 0.752

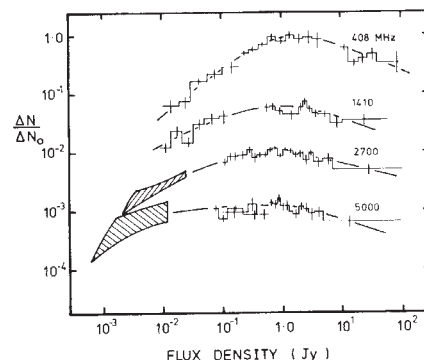


Fig. 1 The differential counts of radio sources at 0.408, 1.4, 2.7 and 5 GHz. The hatched areas are counts inferred from analyses of "confusion" surveys, that is, surveys in which the surface density of sources approaches one per beam area of the radio telescope. The ordinate scale is arbitrary.

and to the possibility of extending the redshift-magnitude relation for galaxies to significantly larger redshifts than before. He was not yet prepared to derive a value for the deceleration parameter from these data. For the quasars, J. N. Bahcall and E. L. Turner (Institute of Advanced Studies, Princeton) showed that despite the enormous scatter, their redshift-magnitude relation is consistent with the predictions of standard world models.

Given this increase in data, what can now be said about the evolution of the radio source population with cosmological epoch? M. Schmidt (California Institute of Technology) showed that for all available complete low frequency samples of quasars and radio quiet quasars the $V/V_{\max}$ test gives consistently values ≈ 0.65 as opposed to the value of 0.5 expected for a uniform distribution. The implication is that the quasars are found preferentially towards the limits of their observable volumes implying a strong evolutionary effect. One intriguing new result, as yet based on rather small statistics, is that the test gives the value 0.5 for flat spectrum quasars selected at high frequencies. If substantiated by improved statistics, this result will be difficult to reconcile with a simple evolutionary scheme because these quasars span a similar redshift range to those found in the low frequency surveys.

The counts of radio sources provide further constraints upon admissible evolutionary models. J. V. Wall (MRAO, Cambridge) showed that the improved statistics of the source counts make it much more difficult to find satisfactory models of the source distribution. All models require the strong evolution of only the most powerful classes of radio sources with cosmo-

logical epoch but it is not yet clear whether an abrupt cut-off to the distribution at large redshifts is necessary. Some of these questions may be answered by deep optical identification surveys of faint radio sources. The greatly increased data base provides new stimulus to the construction of models for the evolution of the radio source population.

There remain all the physical questions of the origin and evolution of radio sources and quasars. Many new facts emerged during the symposium: J. G. Bolton (CSIRO, Parkes) showed how three types of optical quasar could be distinguished on the basis of distinctive radio properties; A. Boksenberg (University College, London) showed the wealth of information contained in high resolution absorption line spectra of large redshift quasars and suggested the lines could be associated with intervening galaxies, a view opposed by E. M. Burbidge and H. E. Smith (Physics Department, La Jolla) who gave reasons for believing them to be intrinsic to the quasars; D. E. Osterbrock (Lick Observatory) described how detailed optical spectral studies of samples of radio galaxies enabled the properties of the emitting regions to be related to those found in other types of active galaxy.

The fundamental problems of understanding the origin and evolution of the radio source population and its significance for the origin and evolution of galaxies are far from being solved. It was clear from the symposium, however, that the impact of radioastronomy upon all aspects of cosmology is enormous. Detailed coordinated programmes of observation at radio and optical wavelengths of all types of radio source are now under way and this fruitful cross-fertilisation is one of the most encouraging prospects for the future of observational cosmology.

Infectivity marker for hepatitis

from Arie J. Zuckerman

INFECTION with hepatitis B virus is associated with the appearance in the serum of hepatitis B surface antigen (originally referred to as Australia antigen) and its homologous antibody. A second antigen, located in the core of the 42–45nm double-shelled spheroidal Dane particle, is intimately associated with the infection and gives rise to the core antibody. These markers have proved extremely valuable in unravelling the epidemiology of hepatitis B

and establishing the global dissemination and importance of this infection (see *Nature*, **247**, 2; 1974), have made possible the routine screening of blood donors for the surface antigen, and resulted in remarkable advances in knowledge of the virology and immunopathogenesis of hepatitis B and its associated chronic liver disease. In addition, the spread of infection can now be controlled in given circumstances, and more recently there have been far-reaching developments which include passive and active immunisation.

But despite the remarkably rapid application of these laboratory findings in clinical practice and preventive medicine a major problem remains unsolved. Hepatitis B virus has not been successfully propagated in tissue culture so that an *in vitro* assay system for infectivity is not available, and in its absence progress towards determining infectivity has been extremely limited. Only two species are known to be susceptible to hepatitis B, man and the chimpanzee. Infectivity cannot, and indeed must not, be experimentally measured in man, although some information can be deduced retrospectively in certain epidemiological settings and as a result of the application of newly developed sensitive serological markers which indicate exposure to the virus. Hepatitis B surface antigen and surface antibody were found in the serum of chimpanzees imported from Africa and living in captivity, suggesting that this species may be susceptible to hepatitis B infection. Experimental transmission of hepatitis B to a small number of previously unexposed chimpanzees established that the disease resembles clinically that in man and that the serological responses are identical. Although chimpanzees will be essential for assessing the infectivity, efficacy and safety of experimental hepatitis B vaccines now under development, there are many practical disadvantages to using them as animal models including their limited availability and expense. The world populations of the apes are already alarmingly small and these animals must be protected. Chimpanzees, although not as rare as the other apes, are being subjected to the combined threats of overhunting and the encroachment of civilisation, and they should be used with the greatest discretion for hepatitis studies (*Wild Hlth Org. tech. Rep. Ser.*, No 512, 1973; No 570, 1975).

Nevertheless, methods for determining infectivity are needed not only for the safety testing and evaluation of prospective hepatitis B vaccines but also for monitoring the removal of infectious virus from blood and blood products, for establishing physical and chemical methods of inactivation of

hepatitis B virus, and, of course, for the identification of infective carriers of the surface antigen of whom there are at a conservative estimate over 110 million (see *Nature*, **250**, 101; 1974).

It now seems that a potentially useful serological marker of relative infectivity may be available. This is a distinct soluble antigen, which although discovered in 1972 by Magnius and Espmark (*J. Immun.*, **109**, 1017; 1972), has until recently received little attention. The antigen—termed *e*—was found in some sera containing hepatitis B surface antigen and it differs immunologically and physicochemically from the previously described determinants of the surface antigen (Magnius, *Clin. exp. Immun.*, **20**, 209; 1975). Paradoxically, antibody against *e* has been found in serum specimens from healthy carriers.

The *e* antigen seems to be somehow intimately associated with the pathogenesis of liver damage. In other studies the *e* antigen was found by the immunodiffusion technique to be significantly more common in patients with chronic liver disease with persistent hepatitis B antigenaemia than in patients with acute viral hepatitis. Furthermore, the *e* antigen seemed to be a valuable prognostic marker since progression to chronic liver disease was recorded by serial liver biopsies in a number of patients with surface antigen-positive acute hepatitis associated with *e*. The clinical significance of the *e* antigen was supported by differences in the clinical, biochemical, and histological findings between patients with the *e* antigen and those without this antigen during the initial phase of viral hepatitis. The *e* antigen has also been found more frequently in surface antigen carriers with histological evidence of chronic liver damage. The presence of *e* antibody, however, is in general associated with only minor histological changes in the liver.

Other observations have also linked the *e* antigen with infectivity. It was commonly found in patients receiving treatment by maintenance haemodialysis and the antigen also appeared in the serum early in the incubation period of acute hepatitis B, at about the time of appearance of the surface antigen and before elevation of the serum transaminases. Furthermore, most of 12 healthy surface antigen carriers who had donated blood without producing clinical hepatitis in recipients possessed *e* antibody, which suggests that when this antibody is present the carriers may no longer be infectious.

Two very recent reports provide additional evidence for the relationship of the *e* antigen with infectivity. Okada and associates (*New Engl. J. Med.*, **294**,

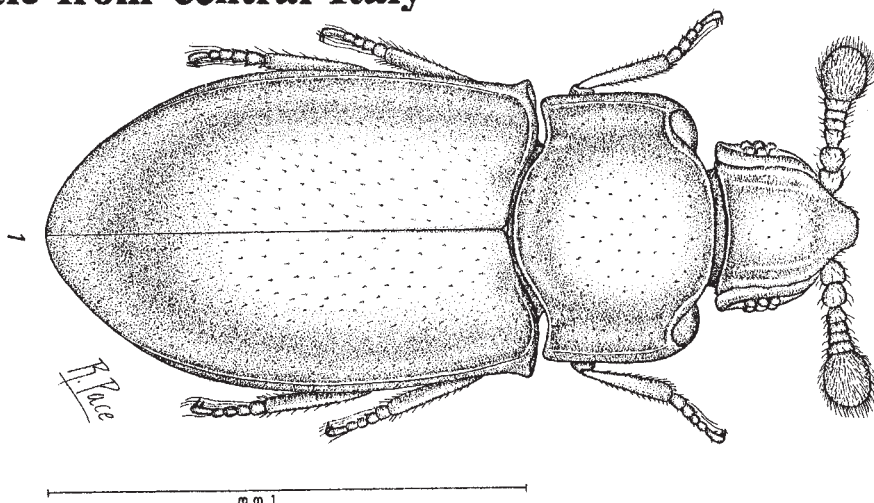
Remarkable new beetle from central Italy

THE coleopterous fauna of Europe is undoubtedly better known than that of any comparable continental area in the world. By this time, to discover even a species new to science is a notable event in the life of a beetle collector; the discovery of a genuinely new genus in western Europe is something very few can hope to achieve. The finder of a new species representing a new taxon at higher than the generic level is almost unique in the last half century.

The fact that this has been achieved by Signor Roberto Pace of Verona, is not purely a matter of good fortune; he has been actively exploring a type of habitat which beetle collectors, except for a few in France, have largely neglected—the deeper layers of the soil. For the lack of an established English term for beetles of this type, we may adopt the French *endogées*. Endogean beetles have a good deal in common with cavernicolous ones—also studied mainly in France—and like them are scarcely represented in the British fauna. *Endogées* are usually small, eyeless, wingless, and more or less depigmented; from the obscurity of their habitat, very little is known about their foods or life cycles.

The insect discovered near Lazio in Central Italy by Pace (*Boll. Mus. civ. St. nat. Verona*, II, 445; 1975) represents not merely a new taxon of at least tribal level, but also a family and suborder hitherto unknown in the modern European fauna—and within the family and suborder represents a new and quite unexpected adaptive type. The suborder Archostemata, to which *Crowsoniella relictata* Pace belongs, is today a very small relict group, to which the oldest known fossils of Coleoptera (of Permian age, see Ponomarenko, (*The Historical Development of Archostematan Beetles*, Moscow, 1969)) have been attributed. Fossils of Archostemata are more or less numerous in "insect beds" throughout the Mesozoic era, but become much rarer in Tertiary deposits, more or less parallel to egg-laying mammals or Dipnoan lung-fishes.

Recent Archostemata are now placed in four small families (Crow-



son, *Boll. Mus. civ. St. nat. Verona*, II, 459; 1975): Ommadidae with one genus and two species in Australia; Tetraphaleridae with two species of *Tetraphalerus* in extra-tropical South America plus *Crowsoniella* in Italy; Cupedidae with four genera and about twenty species distributed through the main continental areas except for Europe and New Zealand; and Micromalthidae with a species in North America and a possibly distinct one in South Africa (Pringle, *Trans. R. ent. Soc. Lond.*, 87, 271; 1938). The larvae are definitely known to be woodborers in *Micromalthus* and a number of species of *Cupes* in America, Japan and Australia, and it is believed that this habit is general in the suborder. Except for *Micromalthus* and *Crowsoniella*, modern Archostemata are moderate-sized beetles with a body length rarely less than 7 mm, with well-developed wings, and active flight has been noted in several species.

Apart from *Crowsoniella*, the only record of subterranean habits in the group is for *Micromalthus*, found breeding in timbers of more or less deep mines in South Africa (Pringle, *op. cit.*). However, species of *Tetraphalerus* have been collected in Argentina (Monros and Monros, *An. Soc. cient. Argentina*, 154, 19; 1952) in areas of bushy vegetation with no large trees, which would seem to be unlikely to furnish the fungus-attacked dead trunks and logs which

are the usual breeding grounds of *Cupes* species; it seems quite possible that *Tetraphalerus* breeds in dead roots underground. If so, the habits of *Crowsoniella* might appear as a natural development from those of *Tetraphalerus*.

The antiquity of the *Tetraphalerus* line is indicated by well-preserved fossils, apparently close to the recent genus, in Jurassic deposits of the USSR (Ponomarenko, *op. cit.*), and the Tertiary occurrence of the type in Europe is shown by the fossil *Tetraphalerites oligocenius* (Crowson, *Ann. Mag. nat. Hist.* (B) 5, 147; 1962) from the Bembridge limestone of the Isle of Wight. The *Crowsoniella* line may well have evolved its flightless and subterranean adults in the Mediterranean region during the Tertiary era. In having antennal grooves above rather than below the eyes, *Crowsoniella* differs from *Tetraphalerus* but may resemble some of the Mesozoic fossils described by Ponomarenko—the separation of the two lines may even be of Jurassic origin. Unfortunately the head is not preserved in the fossil *Tetraphalerites*.

Crowsoniella is surely one of the most ancient elements in the hypogean fauna of Europe; the possibility remains that related types will eventually be discovered elsewhere, e.g. in the Balkan peninsula, or even the Crimea or the Caucasus, if and when systematic search for hypogean forms is undertaken in those areas.

746; 1976) examined for *e* antigen and antibody, serum samples from twenty-three pregnant women who were asymptomatic carriers of hepatitis B surface antigen. Ten mothers with *e* antigen and seven with *e* antibody were identified. Their babies were tested at intervals for over a year after birth. All ten babies born to mothers with the *e* antigen acquired the surface antigen

which persisted throughout the period of observation, and interestingly enough all ten elder siblings of these newborn infants were also found to be asymptomatic carriers. In marked contrast, all seven babies born to mothers with *e* antibody escaped infection with hepatitis B virus and none of their three elder siblings were surface antigen carriers. It was concluded that the

e antigen may be used as an indicator of transmission of hepatitis B virus from carrier mothers to their children. Interesting results were also obtained by Grady and colleagues (*Lancet*, ii, 492; 1976) during a survey of medical personnel accidentally inoculated with blood containing hepatitis B surface antigen. Hepatitis B infection or a surface antibody response occurred in

60% when *e* antigen was detected in the inoculum compared with 31% when *e* antigen was not detectable. The *e* antigen was detected in 74% of inocula accidentally sustained in maintenance renal dialysis and transplantation units, which are recognised hepatitis "high risk" areas, compared with 20% in other clinical units and the resulting incidence of hepatitis was 22% and 6% respectively. It was pointed out that if the correlation of *e* antigen and infectivity is indeed better than indicated by the results of this study, and the correlation is not merely an indirect reflection of some more specifically related phenomenon, then improvements in the sensitivity of the test will be of great value and the potential application to the problems of the transmission of hepatitis B virus will be almost limitless. □

Soil structure discussions in Adelaide

from I. J. Smalley

A meeting of the Soil Physics Commission of the International Society of Soil Science was held at the Waite Research Institute, University of Adelaide, on August 23–27, 1976. The topic was Modification of Soil Structure and the proceedings edited by W. W. Emerson, R. D. Bond, and A. R. Dexter) will be published by John Wiley, London.

AUSTRALIA is the driest continent and it is also the saltiest. This fact causes great problems for Australian agriculture, and for the new town builders at Monarto, particularly as the irrigation water often carries a significant proportion of dissolved salt. But a salty clay can make a good cricket pitch, as J. R. Harris (CSIRO Division of Soils) pointed out in his paper on the maintenance of soil structure under playing turf. Different sports impose widely differing conditions. The soil structure of a cricket wicket requires fine structured clays of high bulk density to be maintained in a condition in which plasticity is retained when wet. When dry, the bounce height of the cricket ball and the ability to withstand intensive playing wear at the surface are important characteristics to match against soil properties. At the other extreme of the soil structure scale, bowling and putting greens are generally constructed on coarse-structured sands where the emphasis will be

on free drainage of surface waters and free rolling of the ball.

Discussion of sporting applications however, occupied only a very small proportion of the meeting; sessions were held on forces between colloidal particles, soil geometry, soil strength, soil tillage, soil management, soil permeability, measurement of aggregate stability, organic matter and aggregate stability, oxides and soil aggregation, organic material and soil structure, modification of soil structure with organic polymers—in all over fifty papers were presented.

The use of soil conditioners (admixtures to preserve a desirable soil structure) was discussed at some length and the meeting was to a large extent a continuation of the annual discussions on soil conditioners (see *News and Views*, 258, 483; 1975). There is growing interest in conditioners as they become more economic and are seen to be effective. Soil conditioning is based on several more or less basic phenomena. One is aggregation of soil particles, which itself depends on adhesion between particles and binding material. In nature, the adhesive product is humus, an ill-defined polymeric material originated by organic matter. Soil conditioners are chemically similar and indeed provide adhesive forces between soil particles. J. P. Quirk and A. M. Posner (Universities of Adelaide and Western Australia) and their co-workers presented recent data on polyvinyl alcohol, particularly with respect to adsorption behaviour on different soil colloids. It seems that there are significant conformational differences between polyvinyl alcohol adsorbed on clay surfaces and on the hydrated aluminium oxide, gibbsite.

Some fundamental studies on soil structure and strength were reported. J. N. Israelachvili and G. E. Adams (Australian National University) have measured the forces between two mica surfaces in KNO_3 solutions. The results exhibit all the essential features characteristic of an interaction involving repulsive double-layer forces and attractive van der Waals forces (see also *Nature*, 262, 774; 1976). R. C. Foster (CSIRO Division of Soils) presented ultramicro-morphological data on some South Australian soils. Soils from irrigated pastures contained large amounts of organic materials, mainly plant remains in various stages of decomposition. Phytoliths were common, but the soil largely consisted of highly compressed and distorted cell wall fragments containing much polyphenolic material. Layers of clay particles down to 0.1 m thick were trapped between the organic layers. Near living roots and also sometimes at considerable distances from them, polysaccharide

mucigels up to 20 μm thick occurred.

J. E. Lloyd (University of Sydney) described a torsional shear box designed to measure the strength of seed beds. The identification of interest of participating engineers and agriculturists was here most noticeably achieved. In general the engineers at the meeting probably learnt more from the agricultural specialists than *vice versa*, but now that the engineers have decided that soil structure at the single particle level is important no doubt they will contribute more and more to this developing subject. □



A hundred years ago

THE obstruction at the entrance to New York Harbour known as Hell Gate was successfully removed by an explosion of dynamite on Sunday afternoon without any of the disasters that many people anticipated. The mass to be removed was about 70,000 cubic yards. The number of borings was 3,500; the number of galvanic batteries 200, placed in an explosion-proof chamber at a distance of 200 feet from Hell Gate. The diameter of the borings was uniformly 3 inches, and the depth varied according to circumstances, from 3 to 11 feet. Fifty thousand pounds of dynamite were used. The shock was not perceptible, not even glass being broken. A vast volume of water and smoke was driven about fifty feet into the air. All the charges were exploded, and the rock is stated to have been thoroughly removed. The explosion was heard at a distance of ten miles, and a tremor like a slight earthquake was heard in New York City and the localities contiguous to Hell Gate. The work has been in progress for seven years.

EARTHQUAKES were felt on the night of September 12–13, at Salonica, and in South Italy, at Reggio. Two motions were observed in the last city, the first one being the most notable, both having taken place on the 13th, between 12 and 1 o'clock, local time. Another earthquake was felt at Salonica, on the 14th, at 5 o'clock in the morning. The Reggio commotions were propagated to Messina and vicinity. They produced quite a sensation, although not destructive.

THE splendid orang-utang in the Berlin Aquarium died last week of consumption. Its friend and playfellow, the chimpanzee, died the next day of consumption and grief. The young gorilla, the one living specimen ever brought to Europe, which we referred to some months ago, is still alive, but ailing. Hamburg not long ago offered 100,000 marks for the gorilla; it is feared that he will soon be sold for less. From *Nature*, 14, September 28, 496, 497; 1876.

review article

Quantum field theory in curved space-time

P. C. W. Davies*

Recent theoretical developments indicate that the presence of gravity (curved space-time) can give rise to important new quantum effects, such as cosmological particle production and black-hole evaporation. These processes hint at intriguing new relations between quantum theory, thermodynamics and space-time structure and encourage the hope that a better understanding of a full quantum theory of gravity may emerge from this approach.

DURING the first quarter of this century, two major revolutions in physical science led to a complete restructuring of fundamental theoretical physics. The quantum theory met spectacular success in describing microscopic phenomena, such as atomic structure and chemical bonding. The theory of relativity, essentially a macroscopic theory, triumphantly explained the known discrepancy in the motion of the planet Mercury, and provided a basis for the understanding of cosmology. In its "special" form, applicable when gravity is negligible but velocities are large, the theory of relativity combines naturally with the quantum theory to yield a rich harvest of new results. The discovery of electron spin and antimatter arose directly out of this synthesis. So too did relativistic quantum field theory, which describes, for example, the interaction of electrically charged matter and electromagnetic radiation. This hybrid theory produced the modern conception of an elementary particle. The quantum field theory version of a particle differs in many respects from the classical notion of a little localised lump of energy. The prototype of the new version is the photon. It is the product of applying quantum theory to the electromagnetic field. Although the word "photon" conjures up the image of a tiny packet of light, this is quite misleading in a crucial respect. What is being quantised is not a localised object, but a mode of the wave field—something spread out over all of space.

The successful reconciliation of special relativity and quantum theory does not extend to the general case, where gravity is strong. Because the latter circumstances usually occur only in macroscopic systems, whereas quantum phenomena are microscopic, this mismatch has not been too serious in practice. In recent years, however, some interesting new possibilities have arisen, in which a proper understanding of quantum phenomena in strong gravitational fields might produce important advances in theory, and even lead to observational consequences.

The long term objective is to construct a full, consistent theory of quantum gravity, with the gravitational field itself quantised, perhaps after the fashion of the quantised electromagnetic field¹. Even after many years of effort, this objective remains as elusive as ever. Sometimes it is conjectured that such theory would only be relevant on the minute length scale of 10^{-33} cm—the so-called Planck length, $(G\hbar/c^3)^{1/2}$ —reducing the quantum gravity programme to a purely academic exercise. It now seems, however, that gravity may well lead to important quantum effects at much larger distances than this.

The basis for this new expectation is a study in which gravity

is treated tentatively as a classical (unquantised) background field, in the presence of which all other fields are to be quantised. This somewhat easier approach is known as quantum field theory in curved space-time, because the unique status of the gravitational field as a geometric entity (the curvature of space-time) has encouraged a geometrical approach to quantum field theory, in the hope of a fresh perspective on formidable problems of principle. These problems always arise when the effects of external classical fields intrude into quantum field theory. It is hoped, though by no means certain, that the background field method represents a consistent first approximation, valid for distances much greater than the Planck length, to a (future) full theory of quantum gravity.

Although only tentative, this semi-classical treatment has nevertheless led to some unexpected discoveries, which may well point the way to a better understanding of the relationship between space-time structure and quantum theory.

Theoretical problems

Before describing those discoveries, it is necessary to explain the radical departures which the introduction of gravity into quantum field theory entails.

First, because the concept of "particle" is a global one, particle processes are sensitive to the global structure of the space-time. Ordinary quantum field theory depends heavily on the simple global properties of flat Minkowski space. Particles are defined successfully with reference to the underlying geometrical symmetries of this space. In a general curved space-time these symmetries are absent, and the notion of "particle" is without obvious meaning. Added to this is the problem that in general relativity, space-time may have new features such as horizons and singularities which must be taken into account in any satisfactory theory of quantum fields in curved space.

In certain simple cases, it may happen that the space-time possesses sufficient symmetry for a natural generalisation of the Minkowski space definition of a particle. This is so if the gravitational field is static, for example^{2,3}. One distinctive state of a quantum field is that in which no particles at all are present. This is called the vacuum state. In Minkowski space the vacuum state is well defined, and corresponds intuitively to what we usually understand as a physical vacuum. In a more general space-time this is no longer true. What may be regarded as a vacuum in one reference frame (system of coordinates) may not be a vacuum in another frame. A very simple and dramatic illustration of this has been given by Stephen Fulling³. The essential features are present even in two-dimensional space-time, a diagram of which is shown in Fig. 1. An observer at

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rest in the (t, x) frame follows the world line A. The other world line (B) corresponds to an observer who undergoes uniform proper acceleration to the right, for all time. His velocity relative to A approaches that of light for $t \rightarrow \pm \infty$. The light rays through the origin are drawn at 45° for convenience. Because information cannot propagate faster than light, these light rays act as an event horizon for B. He can never know about events which occur in the region marked "black hole". This appellation is used because the Universe in the direction of A would grow very dark as B zooms off to the right, on account of an escalating Doppler shift acting on any light travelling from A to B.

The space-time accessible to B is only a portion of the whole Minkowski space. It may be covered by a single coordinate system. In this portion, the space-time is still flat, but differs globally from Minkowski space because of the event horizon. It is a static universe, so that natural definitions of particle states and vacuum exist. Fulling showed that the vacuum state in this portion differs from the usual Minkowski space vacuum. This means that what A regards as a vacuum B does not. This has been interpreted by some⁴ to mean that B will "see" particles, whereas A will not, a somewhat paradoxical idea. There is, however, no reason to suppose that the "particles" which B considers present correspond to "real" physical particles.

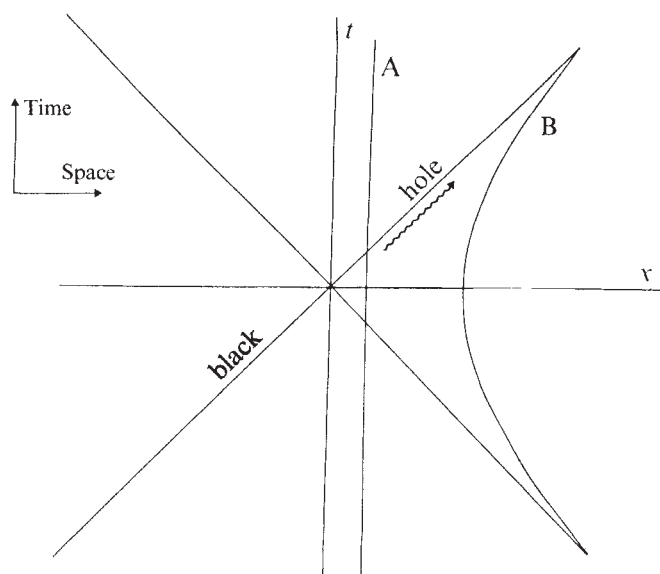


Fig. 1 The observer B undergoes uniform acceleration, while A remains at rest. The light ray through the origin to which B's trajectory is approaching forms an event horizon. B cannot see events above this line. This region of space-time is a "black hole"; light approaching B near this horizon becomes progressively more redshifted by the Doppler effect. The observer B has a completely different description of quantum particle states from that of A, who crosses into the black hole.

Using a simple geometrical argument, it is possible to show⁵ that the spectrum of this mysterious radiation (in the massless case) is a Planck spectrum, corresponding to thermal equilibrium radiation at a temperature of $a/2\pi$, where a is the proper acceleration of the observer B. Recently, William Unruh has analysed the response of a simple model particle detector undergoing uniform acceleration, and confirmed that it does indeed detect thermal radiation at this temperature⁶.

In this simple case, it is tempting to regard the unconventional vacuum as "unphysical". The enigmatic thermal radiation detected by B would then be dismissed as a disturbance caused by the agency which accelerates B. In a general space-time, however there is no easy way to single out one vacuum state over another. Nobody yet knows how to define a vacuum state in the general case.

The stress tensor

The ambiguities inherent in the concept of particles in regions of high space-time curvature has prompted an entirely different approach to quantum field theory in curved space⁷. In this new approach, one deals with mathematical objects which may be defined locally, and not globally as with particles. One important example is known as the stress-energy-momentum tensor, or stress tensor, for short. This object has the additional feature of acting as a source of gravity in Einstein's field equations, so that ultimately one might hope to be able to solve the theory of the coupled classical gravitational-quantum matter fields self-consistently.

Not all the difficulties associated with particle states are thereby circumvented, because the stress tensor must be calculated as an expectation value in some quantum state.

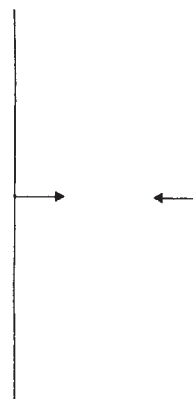
There are, however, several interesting situations in which, at some instant in the past, there exists a well defined vacuum state. The expectation value of the stress tensor can then be calculated in parts of the space-time which are strongly curved, as an expectation value in this initial vacuum state.

The simplest possible such case occurs if one starts with Minkowski space, and then disrupts it by introducing boundaries of some sort. This can be achieved in practice, for the electromagnetic field, using electrically conducting plates or mirrors, which reflect the field at their surfaces (see Fig. 2). If two such plates are held parallel and close, the Minkowski space vacuum is disturbed, even if the plates carry no electric charge. The reason is easy to understand. Modes of the field with wavelength greater than the separation of the plates cannot form as standing waves in the region between them, so they do not contribute to the vacuum energy.

It is easy to calculate the stress tensor between the plates. It was first done by Larry Ford⁸. The calculation does involve one fundamental and far-reaching subtlety, though. Formally, the stress tensor is infinite. This unpalatable circumstance is a result of the so-called "ultraviolet divergence", which is a universal problem in quantum field theory. It arises because even in the vacuum state, each mode of the field with frequency ω still has a zero-point quantum energy of $\hbar\omega/2$. Because the number of modes is infinite, the sum of all this energy is also infinite, progressively greater zero-point energy being contributed by the shorter wavelength modes.

In special relativity, this is no problem, because only differences in energy are observable. And the differences between empty Minkowski space and that with the parallel plates is finite. After subtracting away the divergent part Ford discovered that there was a uniform, static negative energy density between the plates. One consequence of this result is

Fig. 2 The Casimir Effect. Relative to the surrounding vacuum the space between the parallel conducting plates has negative energy, spread in a uniform cloud between the plates. It results in an electromagnetic force of attraction between the plates even though they are electrically neutral and there are no photons present.



that an electromagnetic force of attraction should exist between the mirrors, even though they are electrically neutral. This effect had been known for many years, and is called the Casimir effect. It was predicted in 1948 by H. B. G. Casimir⁹ and has since been measured experimentally¹⁰.

If the mirrors are allowed to move about, a new phenomenon occurs. The static energy density between the plates is augmented by a flux of energy arising from the surface of the moving mirror. In a two-dimensional model, it is possible for the stress tensor to be calculated exactly in terms of functions implicitly dependent on the mirror trajectory¹¹. If the system eventually becomes static once more, particle states may be defined in that future region, and the new phenomenon may be described as particle creation by the moving mirrors. This particle creation is present even for a single moving mirror.

The moving mirror models are useful in that they display in the simplest possible way some of the essential features of how the vacuum state may be disturbed by geometrical effects. When space-time curvature is allowed, similar phenomena occur.

Cosmology

Astronomy provides two scenarios in which gravity is strong enough to stimulate important quantum effects. The first is gravitational collapse, the second is big-bang cosmology.

The pioneering work on the cosmological case was carried out by Leonard Parker in the late 1960s. He showed that particles are created in an expanding universe, though exceedingly inefficiently in present conditions¹². The motion of the universe acts in much the same way as the motion of mirrors, by disturbing the static vacuum. The more violent the motion, the greater the production rate.

During the early stages of the cosmological expansion, the expansion rate was enormously faster than now. At very early times, say 10^{-23} s—long before the Universe had even reached the reasonably well understood lepton era—this production may have been so prolific that it drastically modified the cosmological dynamics. In particular, if the expansion was anisotropic (different in different directions), then the effect of the particle production may have been to “smooth” out the anisotropy. It is a curious unexplained feature that the present condition of the Universe is one of high isotropy. There are hopes that this may now be understood as a result of the back-reaction of quantum effects. Very detailed studies^{13,14} by the Russian astrophysicists Ya. B. Zeldovich, A. A. Starobinski and V. N. Lukashenko encourage these hopes.

It is important to realise that in the curved space case, the particle production occurs out of empty space. It does not arise from sources, or even from moving surfaces. The curvature of space-time itself may be caused by the presence of matter, but this matter does not directly cause the particle production.

Cosmological models have provided important “testing” scenarios for quantum stress tensor calculations (refs 13–16 and unpublished work of J. S. Dowker, R. Critchley, P.C.W.D. and S. A. Fulling). But now a host of new problems arises. Infinite terms which depend on the space-time curvature occur that cannot be removed by subtracting away the infinity which is already present in the Minkowski space case. Some of these terms have the same geometric structure as the left-hand side of Einstein’s field equations, so they can be regarded as simply “renormalising” the values of the Newtonian gravitational constant G or Einstein’s cosmological constant, albeit by an infinite amount. Other infinite terms cannot be so treated, and require a modification of the field equations to accommodate them. On top of all this the finite part of the difference (and perhaps some additional infinite terms) seems to contain ambiguities, which depend on the method by which the infinities are subtracted. There is no doubt that these ambiguities, which are probably inherent in the whole theory, present a major obstacle to understanding the subject. To remove the ambiguities, it is necessary to appeal to principles outside quantum

field theory. Although there is no general agreement on what principles to invoke, very reasonable expressions for the stress tensor have been obtained by these methods (P.C.W.D., and S. A. Fulling, unpublished). For example, it seems certain that there is precisely no creation of photons in an homogeneous, isotropic universe, although there is probably still a non-zero vacuum energy density due to the space-time curvature.

Black holes

Gravitational collapse provides a rare opportunity for the application of quantum field theory because the space-time is approximately flat in the distant region where an observer would be located in practice. For this reason, the asymptotic situation may be described in terms of particles.

A study of this apparently very complicated problem has been carried out by Stephen Hawking¹⁷, with very far-reaching consequences. The collapse, of a spherical star for example, disturbs the initial vacuum of the quantum field, and induces a flux of particles out of the collapsing object. The situation is closely analogous to the accelerated observer shown in Fig. 1. An event horizon forms around the collapsing matter; there is an infinite redshift from the surface. In this case B corresponds to the distant (inertial) observer who watches the collapse. The result is the same. He sees a flux of thermal radiation coming from the black hole. The temperature of this radiation is quite independent of the details of the star or its collapse history; it depends only on the mass M of the star through the formula

$$T = \frac{\hbar c^3}{8\pi G M k} \approx 10^{-6} \left(\frac{M_{\odot}}{M} \right) \text{ K}$$

More complicated expressions have also been derived for rotating and electrically charged black holes.

This astonishing discovery by Hawking has provided an enormous stimulus to the whole subject of quantum field theory in curved space. It also has profound implications in a wider sense. The created particles constitute a radiation flux in the asymptotic region where space-time is flat. This time the flux can, therefore, be unambiguously interpreted as a real flux of energy, coming out of the black hole. It follows that the black hole must shrink as its mass decreases. But as M falls, the temperature rises. Eventually the black hole apparently disappears in an explosive burst of radiation. In practice, only black holes of microscopic size, such as may have been formed early on in the big bang, will have suffered this fate, for a solar mass black hole has a temperature of a mere 10^{-6} K, and quantum effects are negligible.

The fact that black holes have a temperature implies that they are, in some sense, in thermal equilibrium. This means that they may be treated using thermodynamics. Direct analogues of the laws of thermodynamics were already known to apply to black holes even before Hawking’s discovery¹⁸. Now these laws can be placed on a sound footing and new properties of black holes may be discovered¹⁹. For example, it turns out that rotating or charged black holes undergo a thermodynamic phase transition at high values of angular momentum or electric charge. Although it is only a conjecture, many people believe that Hawking’s result heralds the discovery of important new connections between gravity, thermodynamics and quantum theory, with implications far beyond the subject of black holes.

Several enigmas remain. What does an observer who falls into the black hole see? How does the radiation react back on the hole, causing it to shrink? Where exactly is the radiation produced? What happens to the baryons which went to make up the collapsing star, which apparently disappears completely?

To answer these questions it is necessary to know what is happening near, and inside, the black hole, where the space-time curvature is comparable to the relevant radiation wavelengths and the particle concept breaks down. Some useful facts

emerge from a calculation^{20,21} of the stress tensor. So far, this has only been done in two-dimensional models, though the essential features of the Hawking process are still present.

The results show that, in the reference frame of an observer at fixed distance from the centre of the star, the star seems to be surrounded by a cloud of static negative energy. This energy density is sometimes called vacuum polarisation, and is similar to that between the conducting plates in the Casimir effect. It is caused by the static space curvature around the star, and falls off rapidly with distance from its centre. If the star now implodes, a steady flux of energy is superimposed on this static cloud. At great distance this flux is the only contribution to the stress tensor and represents the Hawking radiation. The flux term arises mainly from the surface, and also the interior, of the star. This does not mean however, that particles are created there. Particles are not well defined in this region.

As the star shrinks inside the event horizon, the negative energy cloud around the star can stream into the black hole, causing its mass, and size, to decrease. The precise effect that this has on the collapsing matter is still uncertain.

The separation into flux and vacuum polarisation terms is really somewhat artificial, because it depends on the reference frame used by the observer. If the observer falls into the black hole, the situation is very different. He sweeps through the vacuum polarisation as he falls, so it appears to him no longer a static cloud but an outgoing flux of negative energy. By the time he reaches the event horizon, this flux is comparable in magnitude, but opposite in sign to the Hawking flux. Thus, he does not see much of the radiation which appears to his colleague out at infinity. This is consistent with the analogous situation shown in Fig. 1. There the inertial observer A who, you will recall, sees no particles in Minkowski space, corresponds to the falling observer who enters the black hole.

In practice, the quantum fields which are most important for the black-hole evaporation are the massless ones—electromagnetic, neutrino and, paradoxically, the gravitational field itself. If gravitons exist, they too will be radiated. Calculations²² show that 2% of the luminosity will be due to gravitons, 17%

to photons and 81% to neutrinos. If the black hole rotates, the graviton flux is greatly enhanced. The Hawking effect thus produces the extraordinary situation that graviton processes are comparable in efficiency to electromagnetic processes. Rather than being restricted to the relatively uninteresting scale of 10^{-33} cm it is seen that on the much larger scale of say, 10^{-14} cm, full quantum gravity effects cause significant changes in the behaviour of strongly gravitating systems. These changes could be observed in principle; for example, by measuring the change in the orbital radius around the Sun of a mini black hole of mass about 10^{14} g. The rate of increase around the Sun of the orbital radius would depend sensitively on the mass loss rate, including that due to gravitons.

The new results of the past five years have enormously increased interest in quantum gravity. In spite of its highly esoteric nature, this subject is already seen to provide important new advances in hitherto unrelated subjects, such as black holes, thermodynamics and cosmology. The next five years can be expected to produce more advances still.

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articles

Geology and late Cainozoic lake sediments of the Suguta Trough, Kenya

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A consideration of the geological evolution and late Cainozoic lake deposits of the Suguta Trough in Kenya shows that for the later part of its history the growth of Lake Suguta was probably independent of that of Lake Rudolph, but was influenced instead by climatic change.

THE Suguta Trough (Fig. 1) is the northward continuation of the axial trough of the Kenya Rift. At this latitude the main rift is an ill-defined wide zone of faulting 130 km across. Northwards the trough passes into the southern end of the Lake Rudolph (renamed Lake Turkana) Basin,

and has its structural continuation in the Lake Stephanie Trough¹. At present the Suguta and Lake Rudolph troughs are separated by the Barrier Volcanic Complex. The northern end of the Suguta Trough is an arid area of internal drainage occupied by the saline Lake Logipi and subject to seasonal flooding. The surface of this lake is 118 m below the 1968 level of Lake Rudolph². The Suguta river is the only permanent supply of surface water to the trough, but Lake Logipi is maintained during the dry season by sub-surface flow through the alluvium flooring of the trough.

Only the upper part of the volcanic sequence is described here (Pliocene to Recent); it comprises alternating basalt

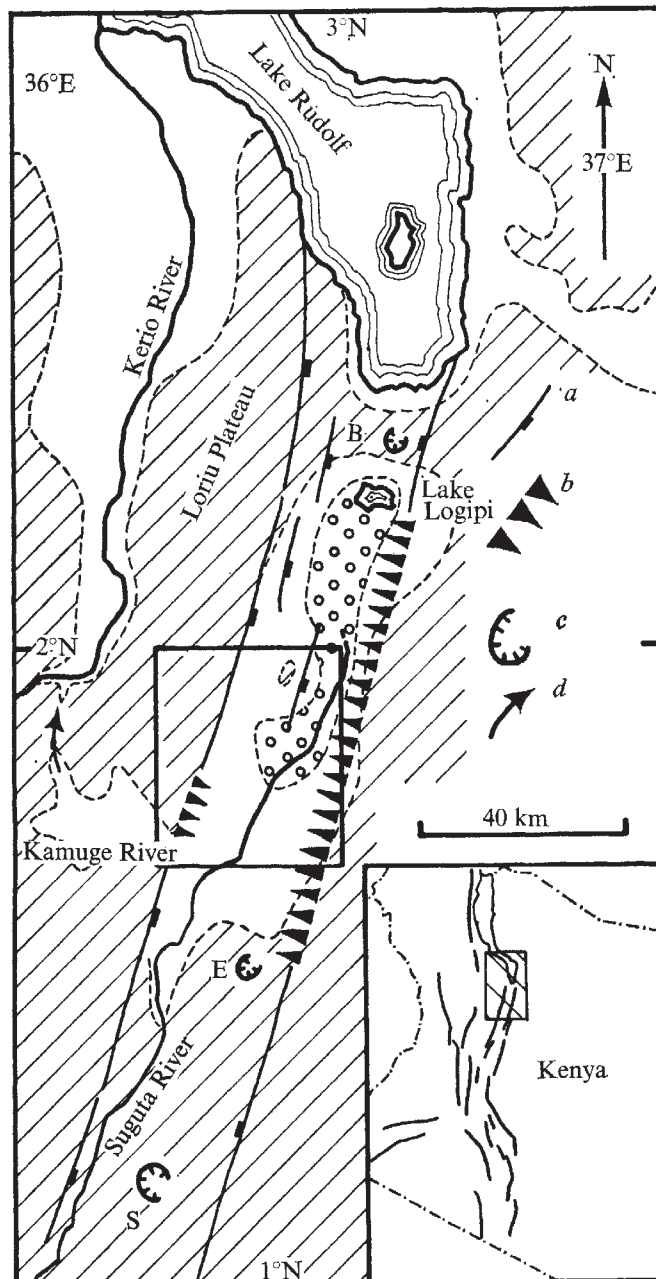


Fig. 1 The Suguta Trough. *a*, Normal fault; *b*, faulted monocline; *c*, caldera volcano; *d*, overflow of Lake Suguta; S, Silali; E, Emurangogolak; B, the Barrier. Open circles, land under 305 m; diagonal rule, land over 606 m. Inset: geographical location of Fig. 1; centred box, Fig. 2.

and trachyte volcanism. The Suguta Trough was first initiated less than 3.0 Myr ago, and was subsequently filled in by younger volcanic products. The reforming of this trough, less than 0.75 Myr ago led to the eventual development of Lake Suguta.

Champion³ has noted the occurrence of sediments flooring the trough and of wave-cut platforms on the southern side of the barrier, and has concluded that the two lakes were continuous before they became separated by growth of the barrier. Once separation was complete, Lake Suguta disappeared because of the insufficient water supply from the Suguta River relative to the evaporation rate. Reconnaissance studies of the Suguta sediments, and preliminary determinations of strand line altitudes, indicate that around 9,600 yr BP Lakes Suguta and Rudolph occupied apparently separate basins. The Recent history of Lake Suguta may have been dependent on climatic change rather

than on an enlarged Lake Rudolph overflowing into the Suguta Trough.

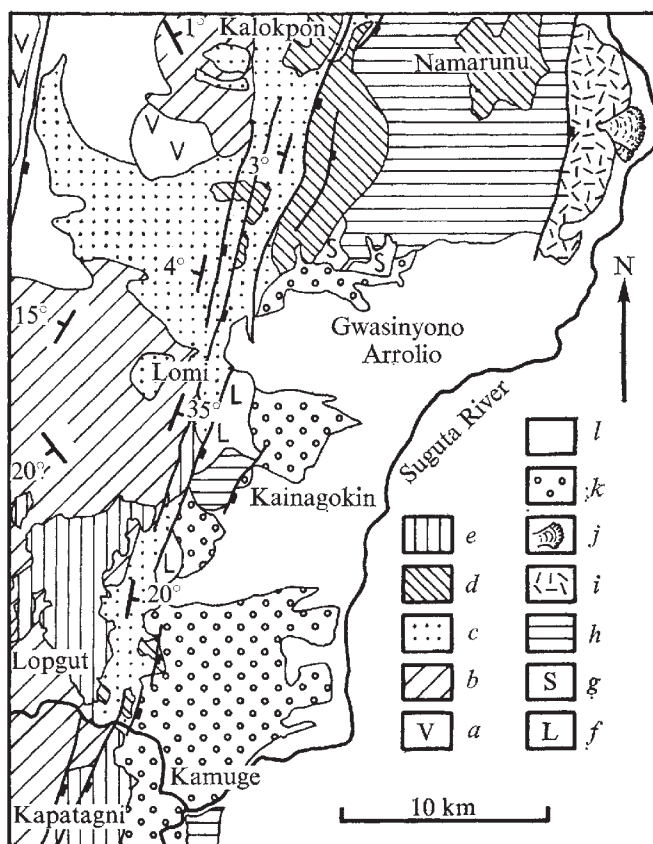
Geological evolution

The geology of the Western Suguta Scarp is shown in Fig. 2.

Nathelot Basalts. These are the Lopiripira basalts of Rhemtulla⁴. A new name for this formation is proposed since the hill of Lopiripira is composed entirely of Nasaken trachyte agglomerate, and the basalts are typically developed at Nathelot. The basalts lie over older, tilted volcanic sequences, and the lava pile has the form of a large, low-angled shield. Nasaken trachytes underlie the Nathelot basalts and have yielded ages largely in the range 5.7–5.4 Myr, but younger dates for this volcano overlap with the age for the basal basalt flows⁵. A single basalt flow from this formation has given an age of 4.5 ± 0.4 Myr.

Loriu Trachyte Group. These trachytes lie conformably over the Nathelot Basalts and comprise three separate quartz trachyte volcanoes (Lopogut, Lomi and Kalakopon) whose alignment parallels that of the Suguta Trough. South of the Kamuge River, the Kapatagne Trachytes occupy a similar stratigraphic position⁴. The trachytes have given ages of 3.0 ± 0.2 Myr (Lomi), 4.4 ± 0.2 Myr (Kalakopon) and 3.3 ± 0.2 Myr (Kapatagne). The Tirr Tirr Trachyte and Basalt Group occurring on the eastern margins of the trough^{6,7}, has been dated at 3.6–3.9 Myr (ref. 8), suggesting it is broadly contemporaneous with the Loriu Trachyte Group. These trachytes are the northern and eastern extension of the South Turkana trachytes of Webb and Weaver⁸.

Fig. 2 Geology of the Western Suguta Scarp. *a*, Nathelot Basalts; *b*, Loriu Trachytes; *c*, Lorikipi Basalts; *d*, Namarunu and Kamuge Trachytes; *e*, Kamuge Basalts; *f*, Suguta Basalts; *g*, Suguta Beds; *h*, member C basalts; *i*, member C pyroclastics; *j*, Recent basalts; *k*, boulder beds; *l*, alluvium.



Monoclinical downwarping of the Loriu and Tiri Tiri groups led to the initiation of a proto-Suguta Trough.

Lorikipi Basalts. This basalt formation erupted from the floor of the trough, infilling and locally overflowing the structure. A single K-Ar determination of 4.0 ± 0.1 Myr cannot be reconciled with the observed sequence.

Namarunu and Kamuge Trachytes. To the north, a mottled green trachyte flow and welded tuff sequence can be traced from the Loriu Plateau to the base of the Namarunu Trachyte Volcano some 300 m lower within the Suguta Trough. These trachytes lie over the Lorikipi Basalts but predate the next phase of faulting. To the south, the Kamuge Trachytes occupy a similar stratigraphic position, and a welded ash flow tuff has yielded an isotopic age of 0.75 ± 0.1 Myr. The Kamuge Trachytes correlate with the lower flows from Emuruangogolak, whose activity commenced before the Suguta Trough reformed.

Kamuge Basalts. These crop out on the Loriu Plateau and are downwarped and downfaulted into the Suguta Trough. A K-Ar determination of '0' Myr indicates the youth of these basalts.

Monoclinical downwarping and minor faulting (between the Kamuge River and Lomi), passing into normal step faults, recreated the western Suguta scarp. The eastern Suguta Scarp was formed by a monoclinical downwarp and associated faulting^{4,7}.

Following this last event, volcanism became restricted to the floor of the Suguta Trough and included the Suguta Basalts, member C basalts, and the continued eruption of Emuruangogolak and the Barrier, although the detailed geology of this complex is not well known. Incision of the Kamuge River system preceded the deposition of the Suguta Beds.

The Suguta Beds

Sediments occurring within the Suguta Trough and post-dating the latest phase of trough formation, have been designated the Suguta Beds. They comprise three sedimentary and one volcanic member (Fig. 3). The Suguta Beds are well exposed and crop out over wide areas of the Suguta Trough floor.

Member A is observed only in the vicinity of Kamuge, and is a dominantly clastic sequence of variable particle size. Rapid lateral facies changes, numerous minor unconformities and washouts indicate a fluvial environment. Member A is overlain with marked unconformity by member B diatomites.

Member B is well displayed at Kamuge and Gwasinyono Arrollo, where it is overlain by member C basalts. A similar sequence is seen at Kainagokin. At Kamuge, an essentially 'pure' diatomaceous sequence is overlain by silts, pebbly silts and silty diatomites, and at Gwasinyono Arrollo, a uniform sequence of bedded diatomites and intercalated pillow lavas occurs. Locally, grits underlie these diatomites.

Members A and B were tilted and downfaulted towards the axis of the trough and subject to erosion before the eruption of member C basalt.

Member C is represented by subaerial basalts resting unconformably on member B. At Gwasinyono Arrollo these basalts are intercalated with basal member D sediments and pass into the more extensive basalt field, capping the Namarunu trachyte volcano. Similar relationships obtain on the northern side of Emuruangogolak. To the east of Gwasinyono Arrollo, and at Kainagokin member C is faulted, although the relationship of the tectonic episode to member D sediments is not clear.

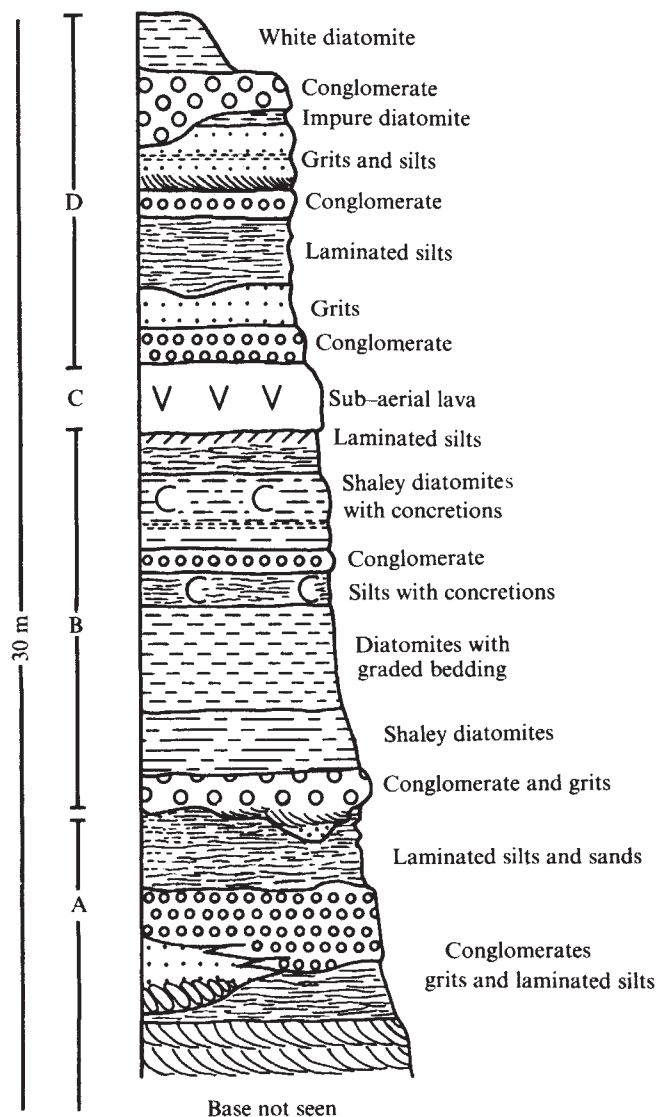
Member D at Gwasinyono Arrollo is a lower clastic sequence which overlies member C and is itself overlain by a thin sequence of well-bedded diatomites. The grain size of the clastic sediments decreases towards the trough axis. Thin diatomaceous deposits overlie member C at Kamuge and Kainagokin. Infrequent diatomites overlie member C basalts on Namarunu at an altitude of around 600 m. They contain gastropod remains, and are associated with algal limestones and wave cut basalt agglutinate cones. The gastropods have yielded a ^{14}C date of $9,660 \pm 210$ yr b.p. (unpublished).

Members B and D record two separate phases of lacustrine sedimentation. During each phase Lake Suguta initially deepened but its development was terminated by desiccation which led to faunal extinction and was followed by subaerial erosion and volcanicity. Remains of *Crocodylus*, *Lates* sp. and, rarely, gastropods (*Melanoides tuberculata*) and bivalves were found within members B and D. Rolled and badly worn bones were noted in the conglomerates of member A. The presence of elements of the Lake Rudolph/Nilotic fauna within members B and D suggests a hydrographic link between Lake Rudolph and Lake Suguta.

Lake levels

It is not possible at present to evaluate the effects of any deformation which may have affected strand lines within the trough. Within the Suguta Trough, high level lake benches occur at altitudes of up to 600 m. The 9,660-yr

Fig. 3 Stratigraphic column of Suguta Beds.



date (member D) is of the same order as the $9,000 \pm \text{yr}$, climatically induced high stand of many East African lakes⁹. On the eastern Suguta Scarp, lake benches have been reported up to 550 m (refs 6 and 7) and up to 575 m on the southern side of the Barrier¹⁰. The age of these benches is not known. Butzer and Thurber reported¹¹ heights of 450–455 m for the highest deposits of the Kibish Formation (Omo area, Lake Rudolph), whose age range (27,444–5,450 yr BP) encompasses the value for member D. Although the chronology of the high level strand lines within the Suguta Trough is not well known it is possible to make some inferences about the relationship of Lake Suguta to Lake Rudolph, based on the assumption that the strand lines of the Suguta Trough have not been significantly deformed.

The differences in altitude between the Lake Suguta and Lake Rudolph strand lines suggest that from member D times at least, the Barrier complex was sufficiently well developed to prevent the two lakes coalescing. The present minimum altitude of the Barrier is 834 m (ref. 10), and it is questionable whether direct overflow of either lake across this complex could have taken place during member D times. An alternative outlet for Lake Suguta could be established by flooding the Kamuge River system, which would overflow into the Kerio river and subsequently into Lake Rudolph. Along the Kamuge river, infrequent diatomites occur at altitudes up to around 600 m. Overflow into the Kerio River would be possible should Lake Suguta levels reach an altitude of around 606 m. The sedimentary record indicates two major cycles in the development of Lake Suguta. This is more consistent with lake growth independ-

ent of Lake Rudolph rather than the progressive desiccation of an arm of Lake Rudolph, cut off by the growth of the Barrier.

Finally, it should be noted that it was apparently the present arid nature of the Suguta Trough which impressed Champion¹. Under the prevailing climatic conditions inflow into the Suguta Trough is insufficient to maintain a large permanent lake, but during the 9,000-yr BP wet phase, Lake Baringo is likely to have supplied increased amounts of water to the Suguta Trough⁹. This increase of overflow from Baringo would further supplement the growth of Lake Suguta, already enlarged by climatic change throughout its drainage system. The rivers of the Suguta system can be expected, during this phase, to have had more permanent flow regimes than at present.

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Significance of major Proterozoic high grade linear belts in continental evolution

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Major Proterozoic high grade linear belts are broadly conformable and sub-parallel to Archaean trends. Some are marginal to Archaean cratons, others intracratonic. All belong to a major set extending across the middle to late Proterozoic supercontinent. They are broadly contemporaneous with hairpin bends of polar wander curves, and some controlled igneous activity. Some are intracontinental structures developed in Proterozoic times when the deeper levels of continental masses underwent internal movement without loss of general coherence.

HIGH grade mobile belts may have formed in the overriding thickened plate on one side of continent–continent collision boundary¹ or they may have developed by reactivation of earlier Proterozoic or Archaean ‘basement’ rocks^{2,3}. Data

relevant to the problem include the lack of offset of earlier oblique structures across several African belts⁴, palaeomagnetic apparent polar wander curves suggesting that the bulk of Africa was an integral crustal unit throughout the Proterozoic^{5,6} and Laurentia in the early Proterozoic (Hudsonian)^{7,8} and divergent polar wander curves that indicate that the Grenville Belt formed at a plate convergent boundary^{7,9} (this is inconsistent with the basement reactivation model¹⁰ and with other palaeomagnetic data¹²). Palaeomagnetic evidence^{1–13} suggests that there may have been a supercontinent throughout Proterozoic times (from 1,000 to 2,200 and possibly 2,700 Myr BP), but this has been challenged¹⁴.

We produce here a plot of high grade Proterozoic mobile belts on the proposed supercontinent (Fig. 1), which, although problematic, is probably more relevant than the Permo-Triassic assembly of Pangaea (Gondwanaland rotated 180° between the Cambrian and the Permian¹⁵). We have not plotted the late Proterozoic Pan-African belts, because

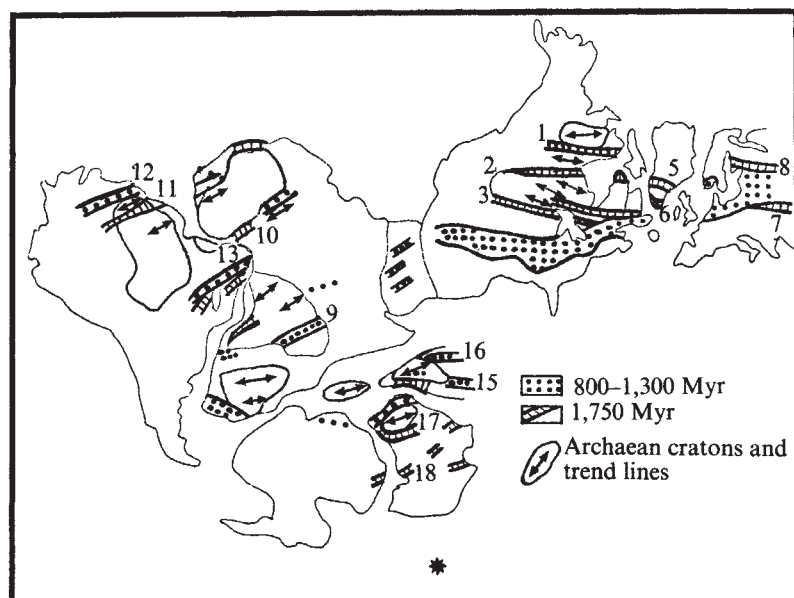


Fig. 1 Map of the mid-late Proterozoic supercontinent, palaeomagnetically determined by Piper¹¹⁻¹³, showing the distribution of two ages of Proterozoic lineaments lying on small circles about a point of rotation (*) off the coast of Australia. Key to the major lineaments: 1, South-east margin of Slave Province^{19,24}; 2, Nelson River, NW Superior Province; 3, SE Superior Province; 4, Grenville Front (and south-west extension)¹⁰; 5, Magssugtoqidian, Greenland (and unmarked Laxfordian of North-west Britain)²⁷⁻²⁹; 6, Ketilidian²⁷⁻²⁹; 7, Central Europe line³⁷; 8, Karelide-Sveccofennide margin³⁸; 9, Karagwe-Ankolan and Kibaran belts of central Africa; 10, Eburnian, east margin of West African craton³⁰; 11, Amazon depression between Guyana and Brazilian Shield³⁵; 12, North of Guyana Shield³⁵; 13, Pre-Mines belts, Brazil^{30,38}; 14, Eastern Ghats, east India^{30,38}; 15, Satpura^{30,38}; 16, Arivalli-Dehli belts, Peninsula India³⁹; 17, Frazer belt^{19,40}; 18, Adelaide belt^{19,40}.

so far they have been determined largely on radiometric evidence¹⁸, nor the many low grade early Proterozoic geosynclines like Circum-Ungava and Hamersley, that contain banded iron formations¹⁷ and probably formed as narrow Red Sea-type rifts according to palaeomagnetic^{7,8} and stratigraphic evidence¹⁸ with possible minor subduction. Neither of these types of belts, both discordant to our general trend, are considered here. In producing our plot (Fig. 1) we are neither attempting to validate the concept of a supercontinent nor denigrating the possibility that plate convergence contributed to the formation of some Proterozoic belts. We do suggest, however, that the sub-parallel distribution of so many belts in Fig. 1 places certain constraints on the movement of plates in the Proterozoic. Our principal aim is to emphasise that the high temperature nature of many of the mobile belts indicates that the continents suffered internal ductile strain under steep geothermal gradients often along transcurrent zones during the Proterozoic¹⁹. The lineaments we discuss could form either in the interior or margin of a thickened overriding plate of a collision-pair or within a large drifting continent or supercontinent¹⁹. Much structural^{4,20} and palaeomagnetic evidence^{5,6} favours the drift during much of Proterozoic time of several large continental sheets that suffered some marginal deformation giving rise, for example, to the Coronation²¹ and Mount Isa²² geosynclines, but more importantly, to internal ductile deformation along many discrete shear zones and mobile belts. Detailed structural studies²³ show that several major shear belts in western Greenland record transcurrent displacements that took place at some depth within a single continental mass. It is likely that this intraplate deformation is a reflection of the ductile nature of much of the Proterozoic continental mass—an intermediate stage between the widespread permobile activity in the Archaean and the more rigid behaviour of plates in the Phanerozoic, when mobile belts were confined to plate margins and internal deformation was brittle.

We use the term lineament here to include shear belts, mobile belts and linear zones of transcurrent displacements of magnetic and gravity anomaly patterns. These are high temperature belts, distinct from low grade geosynclines and high level faults.

Lineament development before 1,300 Myr BP

The lineaments of North America and the North Atlantic region are probably some of the best documented^{19,24}, and together fall into a number of types. Several intracratonic

lineaments strike north-eastwards across the Southern Superior Province and seem to be sub-parallel to the Grenville Belt. These lineaments deflect the Archaean structural, aeromagnetic and gravity patterns of the greenstone belts, and are traversed by undeformed members of the McKenzie Dyke swarm, dated at about 1,200 Myr BP. Some lineaments, particularly the craton marginal set were sites of granite emplacement or basic-acid volcanism during the mid-Proterozoic²⁵. They therefore compare in broad age to the time span of such lineaments in the high grade terrains of the Canadian Shield as the western Superior, south-eastern Slave²⁶, and North Atlantic region. Of these, at least the Nagssugtoqidian of Greenland^{23,27} and the Laxfordian of north-western Britain²⁸ were sites of transcurrent displacements and basic dyke emplacement in late Archaean and early Proterozoic times. Both these lineaments became the junctions between regions stabilised about 2,700 Myr BP and younger Proterozoic mobile belts²⁹. In the later development of both lineaments, displacements perpendicular to them accompanied by granitic intrusions became prominent by about 1,600 Myr BP. It may be that this later stage is expressed in the Ketilidian Mobile Belt, which is dominated by displacements along the craton margin, and by considerable granite emplacement²⁷. When plotted on a reconstruction of the continents before continental drift, the lineaments at the margin of the North Atlantic region, including those of eastern Scandinavia, fit the directions of both the intracratonic and marginal lineaments of the Canadian Shield^{19,28}. Our argument is supported by the general coherence of palaeomagnetic poles from the Canadian Shield, which suggest that internal blocks did not move by large amounts relative to one another during the Hudsonian Orogeny when the shield seems to have undergone internal lineament deformation without loss of general coherence^{7-9,14}.

Early Proterozoic linear mobile belts are widespread in the Gondwana group of continents^{11,19} and are mainly of the craton-marginal type. In Australia they form the junctions between the Yilgarn and Carpentaria blocks. Near-parallel structures occur in Antarctica, and the Eastern Ghats of eastern India. The Eburnian Belt lies along the eastern margin of the West African Craton and has a possible extension in South America, north of the Guyana Shield. The Ubendian and the older Limpopo belts in central and southern Africa are, however, oblique to this general trend.

The early Proterozoic lineaments seem to share a common evolution. Most probably they originated in the late

Archaean or early Proterozoic as transcurrent dislocations with associated basic dyke swarms which in several lineaments could fit the regional pattern. The development of the lineaments seems to have been arrested with the cratons at this early stage. Further development occurred in the lineaments along the margins of the cratons. In several lineaments of the North Atlantic Province a change from transcurrent displacements to movements perpendicular to the craton margins³⁷ was marked by an increase in granitic emplacement both along the lineaments and in broader zones parallel to them within the larger mobile belts. The culmination of mid-Proterozoic mobile belt development is probably marked by the intrusion of anorthositic, alkali complexes and rapakivi granites marking a major belt extending from the western USA to the Urals, which ceased not long after 1,300 Myr ago²⁵.

Lineament development between 1,300 and 1,000 Myr BP

Although the development of the last group of lineaments seems to have been arrested before 1,300 Myr BP, a number of narrow zones of basement reactivation and wider belts of tectonism and igneous activity developed between 1,300 and 1,000 Myr BP (ref. 30). The Grenville Belt and its possible extensions in North America and Europe is wider and more extensive than all other lineaments. Palaeomagnetic data¹² cast doubt on the concept that the formation of this belt involved some lateral plate motion⁷⁻⁹.

A number of linear zones of this broad age can be outlined as anomalous structural, igneous and radiogenic features in Gondwanaland. These form a set of parallel lineaments concordant with the line of the contemporaneous Grenville Belt projected as a major zone extending through western Arabia, central Africa and along the eastern coast of South America (see Fig. 1), and with most of the earlier lineaments dating from around 1,700 Myr BP. Some belts, for example the Aravalli-Delhi Belt were active in both periods. The development of these 1,300–1,000 Myr lineaments has much in common with the later stage of development of the earlier lineaments, particularly in the association of major tectonism, the basic dyke emplacement about 1,200 Myr ago, and the syn- and post-orogenic granite emplacement and widespread thermal events.

Discussion

These Proterozoic lineaments seem to be coherent, global-scale, structures which belong to a single, broadly conformable pattern for about 1,000 Myr. Major events in the evolution of each age group of lineaments seem broadly contemporaneous with the hairpin bends of the single polar wander track for North America and Africa at 1,700 Myr BP and 1,300–1,000 Myr BP (refs 11 and 31), and therefore seem related to major changes in position of the Proterozoic continents or supercontinent. Many lineaments evolved as transcurrent dislocations within formerly continuous continental crust, rather than as sutures marking closed oceans.

In the context of a single Proterozoic supercontinent¹¹⁻¹³ both age groups of lineaments fit as zones of transcurrent displacement which lie on small circles about a point of rotation off the Australian part of the early continent. This agrees broadly with the interpretation of the Coronation²¹ and Mount Isa²² geosynclines and the Belt-Purcell Series of western North America as continental margins developed on the opposite leading edges of the rotated supercontinent.

Following the formation of the belt of anorthositic and rapakivi granites, the culmination of lineament development was expressed by late Proterozoic rifts, as in the Gardar Province of southern Greenland, which controlled lava extrusion, clastic continental sedimentation and the intrusion of alkaline complexes. These rifts may also be seen as the earliest expression of continental breakup connected

with the eventual formation of the Proto-Atlantic Ocean in late Proterozoic times; they are comparable with the Triassic rifts containing continental sediments and volcanics along the Atlantic coast of the USA³². In fact, the large number of failed rifts and aulocogens of late Proterozoic age³³ is probably a demonstration of how difficult it was to fragment the Proterozoic supercontinent. Both types of Proterozoic lineaments are, on a global scale, sub-parallel to the trends of the Archaean granite-greenstone belts and therefore could be influenced by the overall Archaean structural pattern³⁴. A few Proterozoic lineaments seem to have incidentally influenced the form of some Phanerozoic orogenic belts (for example, the old Amazon depression shield lineament segmenting the Andes along the Amotape Zone³⁵).

Besides simple collision, there are several other means of explaining the formation of different Proterozoic high grade belts. Evidence from the shear belts is consistent with, but does not necessarily suggest, the idea that they are the deep-seated equivalents of modern intracontinental transform faults³⁶. The Grenville Belt has been considered as the result of the slow drift of a continental plate over one or a series of hot spots, giving rise to successive stages of extension and compression. At deep levels of erosion an aulocogen may appear as a high grade ensialic mobile belt with some degree of transcurrent movement⁴². Upwelling and diverging convection currents may give rise to high heat flow and the formation of ensialic belts⁴³.

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Sex hormone receptors in mammary tumours of GR mice

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Studies of the growth behaviour and hormone receptor contents of GR-mouse mammary tumours suggest that the hormone responsive tumours are mixed populations of hormone-dependent cells and autonomous cells. The hormone-dependent moiety of these tumours contains high levels of progesterone receptor and oestrogen receptor. The autonomous moiety has a low but probably significant oestrogen receptor level, and is practically devoid of progesterone receptor. Androgen receptor levels in both moieties are low. Endocrine ablation prevents growth of the hormone-dependent moiety of the tumours, but not of the autonomous moiety.

In approximately one-third of patients with metastatic breast cancer, the tumour responds favourably to ablative or additive forms of endocrine therapy¹. Several groups of investigators have shown that a relationship exists between the presence of specific oestrogen-binding proteins in human breast cancer tissues and hormone responsiveness of the tumour²⁻⁴. But although assays of oestrogen receptors have important prognostic value, a large

proportion of the oestrogen receptor-positive group does not respond to endocrine treatment⁵. Similarly, studies on DMBA-induced mammary tumours in rats suggest that determination of cytoplasmic oestrogen-binding capacity alone does not perfectly predict whether the growth of a tumour will be affected by ovariectomy⁶.

It has been proposed that in non-responding receptor-positive cases the oestrogen receptor can bind hormone but cannot subsequently cause gene activation, and that assays of progesterone receptor may have greater prognostic significance⁷. It has also been suggested that determination of androgen receptor may be an important tool for predicting response to castration in premenopausal patients⁸.

To evaluate the roles of different hormone receptors in mammary tumour growth, it is important to have available model systems with which the influence of each factor can be established. The GR-mouse system seems to be useful in this respect. Hormone-dependent mammary tumours can be induced in ovariectomised GR mice by continuous treatment for 3-4 months with oestrone and progesterone. The tumours can be transplanted into castrated (O20×GR)_{F1} hybrid mice which are treated with oestrone and progesterone. The tumours generally retain their hormone dependence during the first transplant

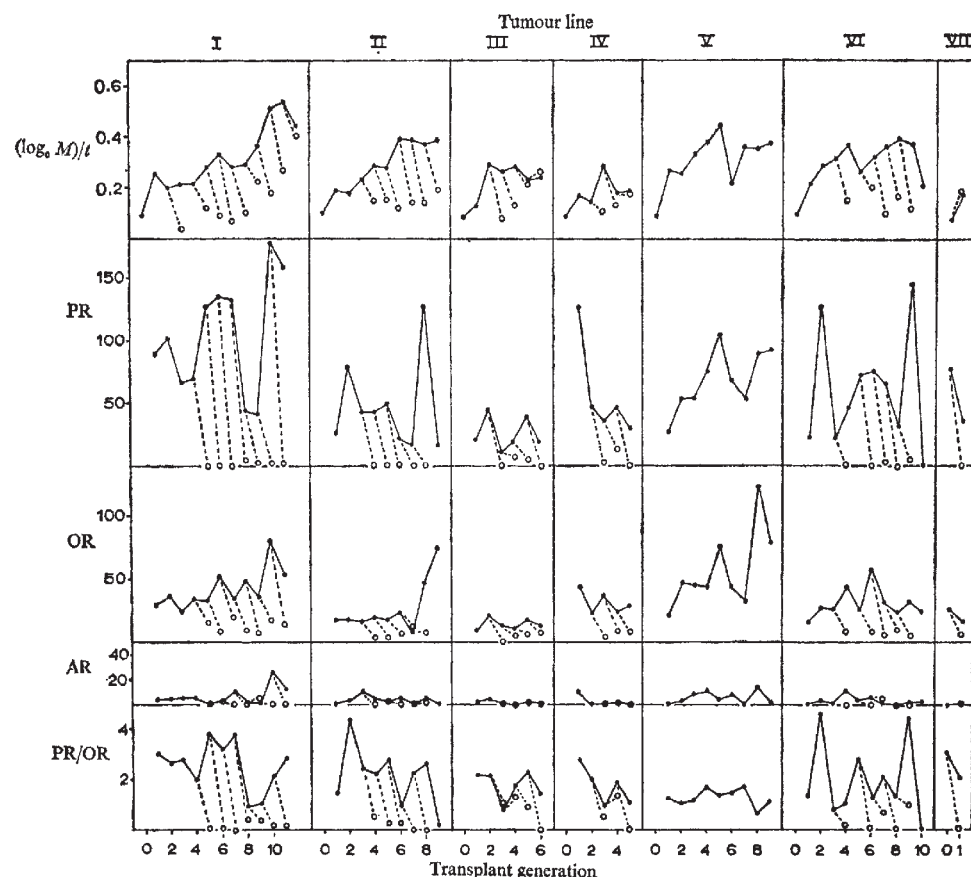


Fig. 1 Serial transplantation of mammary tumours in castrated GR mice (—○—), and in castrated GR mice which were treated continuously with oestrone and progesterone (---○---). M is the tumour weight (mg) and t the time (d) after grafting. Receptor contents of the tumours are expressed in fmol per mg cytosol protein. Receptors of progesterone (PR), oestrogen (OR) and androgen (AR).

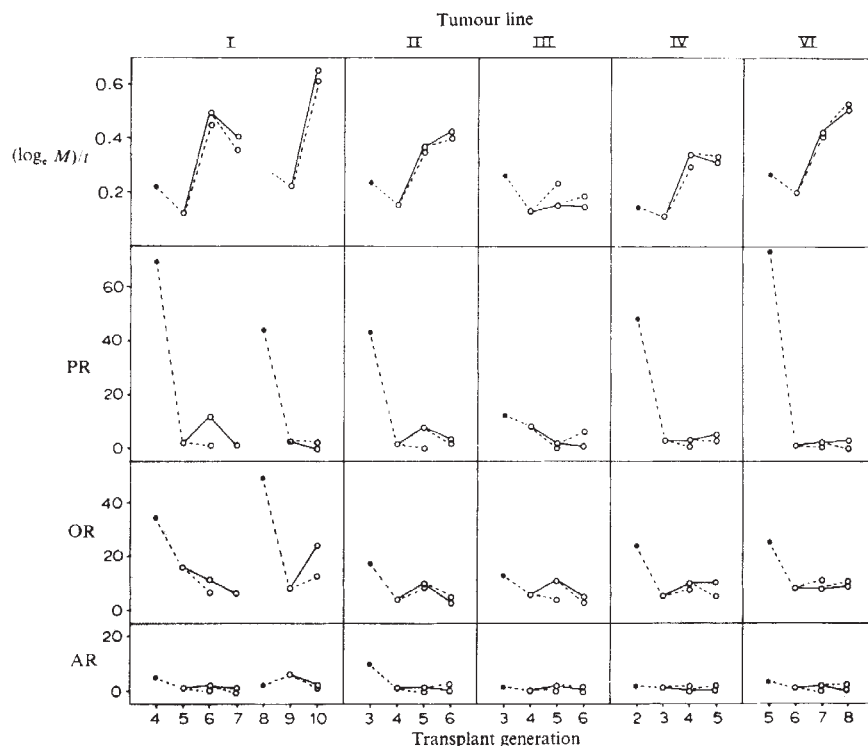


Fig. 2 Serial transplantation of mammary tumours in castrated GR mice (—) and in castrated GR mice treated with oestrone and progesterone (---). M is the tumour weight (mg) and t the time (d) after grafting. Receptor contents of the tumours are expressed in fmol per mg cytosol protein. Receptors of progesterone (PR), oestrogen (OR) and androgen (AR).

generation, but in subsequent transplant generations a progressive decrease in hormone dependence is observed which eventually leads to complete hormone independence. An advantage of using this system is that a certain tumour can be studied in various endocrine conditions. We have reported⁹ previously that the mammary tumours which respond to oestrone and progesterone contain higher levels of oestrogen receptor than the autonomous mammary tumours. We now present results of a study on the relative concentration of oestrogen receptor (OR), progesterone receptor (PR) and androgen receptor (AR) in these tumours, and the relationship with tumour growth behaviour.

Transplantation series

Mammary tumours were induced in ovariectomised GR mice and tumour lines developed by serial transplantation from seven separate primary tumours (tumour lines I–VII). The degree of hormone responsiveness of the primary tumours and of each transplant generation was tested by grafting portions of the tumours in two castrated mice which were treated with oestrone and progesterone (OPC mice), and two castrated mice which were not treated with hormones (C mice). Tumours which were transplantable in OPC mice but which did not yield visible outgrowths within 3 months in the C mice were designated hormone dependent (HD). Tumours which were transplantable in both cases but which yielded outgrowths in OPC mice sooner than in C mice, were designated hormone responsive (HR). Tumours which grew equally well in OPC as in C mice, were designated autonomous or hormone independent (HI).

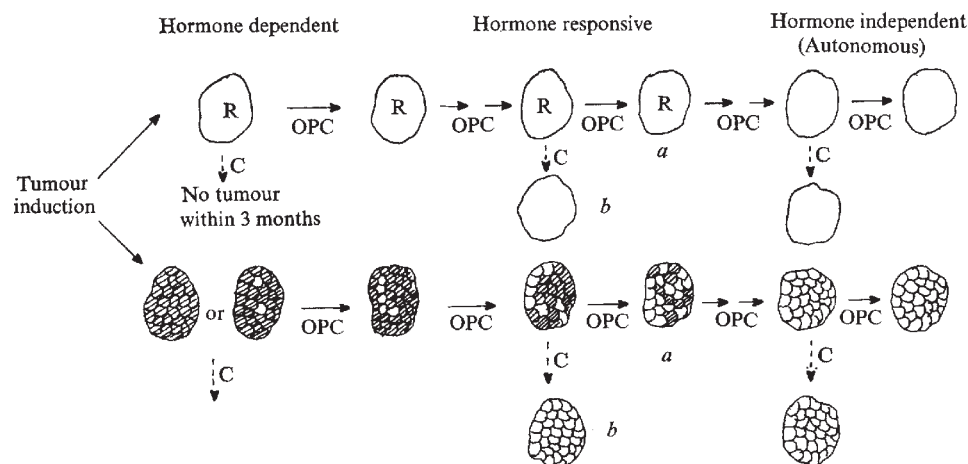
Samples of 500-mg tumour tissue were used for hormone-receptor assay. 2,4,6,7-³H-Oestradiol (85 Ci mmol⁻¹) was obtained from the Radiochemical Centre. R5020 (17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione), ³H-R5020 (51.4 Ci mmol⁻¹), R1881 (methyltrienolone) and R1881-6,7-³H (58.2 Ci mmol⁻¹) were gifts of Dr J. P. Raynaud, Roussel-Uclaf. Nafoxidine hydrochloride (U 11,100A) was a gift from the Upjohn Company. Determination of oestrogen receptor in the tumour cytosols was carried out with the dextran-coated charcoal assay^{10,11}. The same method using ³H-R5020 was used for progesterone receptor assay¹², and with ³H-R1881 for androgen receptor assay¹³. Controls contained excess non-radioactive ligand. Protein was assayed according to Lowry *et al.*¹⁴. Recep-

tor levels were expressed as fmol mg⁻¹ cytosol protein. Tumour tissues stored at -70 °C were used for receptor assays, since in cytosol preparations sometimes losses of OR and PR were found to occur up to 0.2 and 0.8% d⁻¹, respectively, when these were stored at -70 °C.

Figure 1 shows the results obtained with transplantation series I–VII. As a parameter of the growth of each individual tumour we calculated the ratio $(\log_e M)/t$, where M was the weight (mg) of the tumour when it was collected (usually about 3,000 mg), and t was the time (d) after grafting. Six of the primary tumours were found to be HD, since they were transplantable in OPC mice but not in C mice; transplantation series I–VI were developed from these tumours. The seventh primary tumour was found to be autonomous, since its first transplant generation appeared equally rapidly in OPC mice and in C mice; this line (tumour line VII) was therefore discontinued. Tumour lines I–IV and VI exhibited a decrease in hormone dependency after the second to third transplantations, but line V remained HD up to the ninth transplant generation.

When HR tumours were grafted in C mice (Fig. 1, dotted lines), they gave rise to tumours with markedly lower $(\log_e M)/t$ values than when they were grafted in OPC mice (Fig. 1, solid lines). This was due to the fact that in the C mice the tumours appeared after a longer lag period than in the OPC mice. The tumours which appeared in C mice had significantly lower PR and OR contents than those which were obtained in OPC mice. Evidence that the tumours derived from C mice were autonomous was obtained by transplanting portions of 6 of these tumours in OPC mice and C mice. As is shown in Fig. 2, oestrone and progesterone did not cause an increase in the $(\log_e M)/t$ values of the transplants. Furthermore, the tumours remained HI during subsequent serial transplantations, whether or not hormones were given, and their receptor contents remained low.

These results indicate that in the absence of oestrone and progesterone, the HD cells in a hormone-responsive graft did not multiply, and that the HI cells in the graft consequently gave rise to autonomous outgrowths. The long lag period can be attributed to the long time required for the small percentage of HI cells in the graft to form a visible tumour (Fig. 3b). This suggests that all tumours indicated in Figs 1 and 2 by open circles are autonomous.



the presence of hormones (a) both the hormone-dependent cells and the autonomous cells can multiply, whereas in the absence of hormones (b) only the autonomous cells can multiply. Case b therefore yields an autonomous tumour with low receptor values. It is not clear whether the primary hormone-dependent tumour from which the transplantation series is derived is entirely devoid of autonomous cells or already contains minute amounts of autonomous cells. Both alternatives are illustrated at the bottom left.

Receptor levels and ratios

Figure 4 shows the average values and standard deviations of receptor contents of hormone-dependent, hormone-responsive and autonomous tumours. The levels of PR and OR were both markedly lower in HI tumours than in HD or HR tumours. No significant difference in hormone-receptor content was found between HD and HR tumours. The results indicate that PR levels below 17 fmol per mg cytosol protein and OR levels below 14 fmol per mg cytosol protein were markers for hormone independence. Androgen-receptor levels in HD and HR tumours were markedly lower than PR and OR levels in these tumours. AR levels in HD and HR tumours were on average slightly higher than in HI tumours. Figure 4 also shows the results obtained when the PR/OR ratio of each tumour was calculated separately, and the average values and standard deviations of the data were computed. HD and HR tumours had significantly higher PR/OR ratios than HI tumours. No significant difference in PR/OR ratios were found between HD and HR tumours. The results indicate that PR/OR values smaller than 0.8 were markers for hormone independence.

Discussion

Calculation of PR/OR ratios served as an additional check for the significance of absolute PR and OR values. A suitable marker for hormone independency in GR-mouse mammary tumours seemed to be PR and OR contents lower than 17 and 14 fmol per mg cytosol protein, respectively, together with a PR/OR ratio lower than 0.8. It should be pointed out that these values apply to the method used for receptor assay in which only the number of free receptor sites was determined. In the OPC

mice the endogenous levels of both progesterone and oestrogen would be very high, so the quantitative levels of free cytosol receptors could be small compared with the bound receptor.

At present it is not clear whether the level of cytoplasmic hormone receptor can be taken as a measure of the number of cells in the tumour that contains the receptor. To substantiate this conclusion, other methods such as autoradiography should be utilised. If this assumption is correct, however, our data are consistent with the assumption that hormone-responsive mouse mammary tumours are heterogeneous and that they contain the following cell populations. (1) Hormone-dependent cells with average PR, OR and AR contents of 74, 48 and 6 fmol mg⁻¹ cytosol protein, respectively, and an average PR/OR ratio = 2.0. These cells depend for growth on oestrogen and progesterone. The hormone-dependent population might consist either of one cell type with all three receptors, or might contain different cell types with different receptor contents. There is evidence for the presence of different stem lines in hormone-dependent rat mammary tumours¹⁵. (2) Hormone-independent (autonomous) tumour cells with average PR, OR and AR contents of 3, 9 and 1 fmol mg⁻¹ cytosol protein, respectively, and an average PR/OR ratio = 0.4. These cells are not affected by endocrine ablation.

The results obtained with the GR-mouse model system reveal some of the limitations of hormone-receptor assay as a parameter on which to predict whether the growth of a mammary tumour will be affected by endocrine treatment. One of these limitations is that whereas low PR, OR and PR/OR values seemed to be a good indication for hormone independence, high PR, OR and PR/OR values did not reveal whether or not the tumours contained autonomous cells in sufficient quantity to

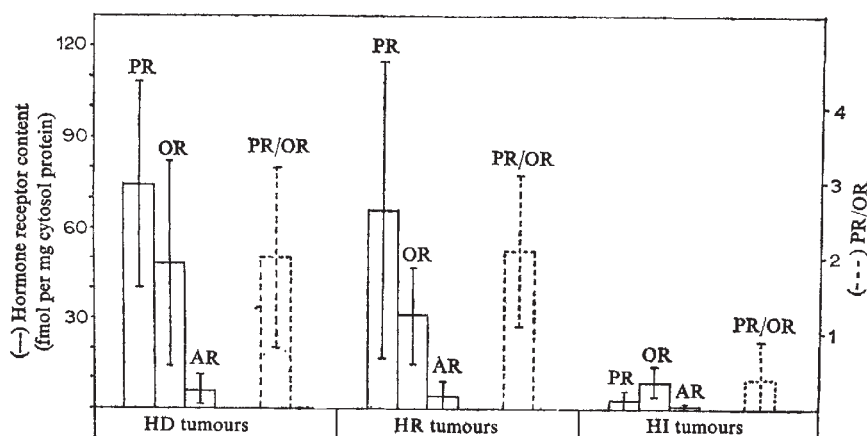


Fig. 4 Hormone-receptor contents of mammary tumours of GR mice. Receptors of progesterone (PR), oestrogen (OR) and androgen (AR). Receptor levels are expressed in fmol per mg cytosol protein. PR/OR, progesterone receptor/oestrogen receptor content ratio (mean $\pm$ s.d.). Hormone-dependent (HD), hormone-responsive (HR) and hormone-independent (HI) tumours.

contribute significantly to growth behaviour. Thus no essential difference in receptor levels was found between HD tumours and HR tumours (Fig. 4). Furthermore, tumours which became autonomous after serial transplantation in OPC mice usually still had significantly higher receptor contents than autonomous transplants derived from C mice (Fig. 1).

Variations in sampling also impose limitations on the significance of receptor assays. We often found marked differences in receptor values between different samples taken from one hormone-responsive tumour. If these reservations also apply to hormone-responsive breast cancers in human patients, they might contribute to the high percentage of clinically defined non-responding positives.

The finding that autonomous mammary tumours of GR mice generally had small but significant amounts of OR, but practically no PR, is of interest. Since in oestrogen target tissues the synthesis of PR depends on the action of oestrogen¹⁶, it seems possible that in some autonomous mammary tumours of GR mice the lesion is at the nuclear level. For instance, this might be the case with some autonomous tumours of transplantation series I which had OR levels about equally high as some hor-

mone-responsive tumours of other lines (Fig. 1). This possibility has, however, to be investigated further.

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Rotational diffusion of band 3 proteins in the human erythrocyte membrane

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Band 3 proteins rotate in the human erythrocyte membrane; rotation is almost certainly confined to an axis normal to the plane of the membrane and is characterised by a diffusion coefficient of the order of $1,000\text{ s}^{-1}$. Spectrin does not restrict rotational motion of band 3 proteins.

MANY, and probably most, cell membranes contain lipids arranged in a bilayer configuration. According to currently popular concepts of membrane structure, 'intrinsic' or hydrophobic membrane proteins are essentially solutes in the fluid lipid bilayer¹. As such they would be expected to undergo free diffusion—that is, diffusion the rate of which is determined only by the size of the protein and the 'viscosity' of the membrane. There is good evidence that this is the case for rhodopsin where Cone^{2,3} has measured both lateral and rotational diffusion constants. In other instances there is qualitative evidence that proteins diffuse rather rapidly in the plane of the membrane (for reviews, see refs 4–6). On the other hand, at least one membrane protein, bacteriorhodopsin, is completely immobilised by protein–protein interactions^{7–9}. At the present time, it is by no means clear to what extent proteins do diffuse freely and to what extent free diffusion is restricted by interactions with other components of the membrane, or conceivably of the cell—for example, microtubules or microfilaments.

To investigate this question, it is necessary to have available a technique which measures diffusion quantitatively and can be applied generally. In the case of rotational diffusion of membrane

proteins, rotational motion characterised by relaxation times at least as slow as microseconds has been both predicted and demonstrated^{2,6}. This causes an experimental difficulty since such a time range is not adequately covered by established techniques of measurement. Indeed, investigation of the rotational diffusion of membrane proteins has only so far been possible with a very limited number of proteins which possess special spectroscopic properties^{2,5–7}.

To overcome this difficulty, we have developed a new technique for measuring slow rotational diffusion, which exploits the long lifetimes of triplet states of organic molecules^{7,10,11}. Using the method of flash photolysis, triplet (or other long lived) states may be detected by transient absorbance changes after flash excitation. If the exciting light is plane polarised, the absorbance changes are in general dichroic. The rate of decay of dichroism gives information about the rate of rotational motion of the excited molecules.

Since most proteins do not naturally possess a suitable chromophore for such measurements, it is necessary to attach an artificial chromophore, or probe. Eosin (2,4,5,7-tetrabromofluorescein) is a favourable choice of probe since the four bromine atoms promote intersystem crossing. Covalent coupling to proteins may be achieved using the reactive isothiocyanate derivative (eosin–NCS). From studies with protein–eosin conjugates dissolved in viscous solutions, we have concluded that reliable measurements of rotational relaxation times in the microsecond–millisecond time range may be made using the flash photolysis method^{10,11}. Here we report the first application of the method to proteins in a cell membrane.

The human erythrocyte membrane was chosen for these studies because the composition and properties of its proteins are relatively well characterised^{12–14}. Major constituents are spectrin, a fibrous protein composed of two polypeptides of

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molecular weights 220,000 and 240,000, and band 3 (otherwise known as component *a*). Band 3 consists of hydrophobic proteins which span the membrane, have a molecular weight of about 90,000 and comprise about 24% of the total membrane protein¹². They are implicated in anion and other transport functions¹⁵⁻¹⁸. The major sialoglycoprotein, glycophorin, also spans the membrane and has been extensively characterised¹⁴. It is thought that glycophorin in association with band 3 constitutes the membrane particles revealed by freeze-fracture electron microscopy^{19,20}.

Labelling of human erythrocytes with eosin-NCS

Red blood cells obtained from fresh or recently outdated human blood were washed three times with 310 mosM sodium phosphate buffer, pH 7.4. Packed cells (15 ml) were incubated with 3 mg eosin-NCS for 3 h at 22 °C. The cells were then washed twice more with isotonic buffer to remove any unreacted label and subsequently haemolysed in 40–50 volumes 20 mosM sodium phosphate buffer, pH 7.4. The ghosts were sedimented by centrifugation for 20 min at 20,000*g* and washed three to four times with 20 mosM buffer. All operations except the labelling step were carried out at 0–4 °C.

The amount of bound eosin was determined spectrophotometrically as described previously^{10,11}, after first solubilising ghosts with SDS. Protein was determined using the method of Lowry *et al.*²¹. Typically the ghosts contained 0.5–1.5 µg eosin per mg protein.

The proteins labelled by the above procedure were identified using SDS–polyacrylamide gel electrophoresis and by selective extraction experiments (details presented elsewhere). Briefly, SDS–polyacrylamide gel electrophoresis was carried out in a manner similar to that described by Fairbanks *et al.*²². Using gels of dimensions 0.85 × 8 cm loaded with 0.5 mg protein it was feasible to locate eosin fluorimetrically. Eosin fluorescence was located predominantly in the region of band 3. A small amount of eosin was also associated with bands 1 and 2 (spectrin). There were negligible amounts of eosin bound to other proteins. Using gels made from solutions containing 7.5% acrylamide (w/v), 2.73% *bis*-acrylamide (with respect to acrylamide), it was possible to separate glycophorin from band 3. In this way we could demonstrate that little or no eosin was bound to glycophorin.

These results were further confirmed using selective extraction procedures. Selective extraction of bands 1, 2 and 5 by incubation at low ionic strength in the presence of EDTA²³, demonstrated that less than 10% eosin was associated with these components. On the other hand, extraction with Triton X-100 (ref. 23), which has partial selectivity for band 3, released up to 80% eosin from the membrane. Finally the labelled ghosts were extracted with chloroform–methanol (2:1) as described by Hamaguchi and Cleve²⁴. The amount of eosin extracted with lipids into the organic phase was negligible. A few per cent eosin appeared in the aqueous phase together with the membrane sialoglycoproteins.

The selective labelling which we have observed is in accord with other authors who find that only band 3 and sialoglycoproteins are available at the outer surface of the red cell^{12, 25-27}. Since some of our label is associated with spectrin, we conclude that there is some permeation of eosin-NCS through the membrane. We found little labelling of glycophorin, and a similar observation was made by Cabantchik and Rothstein¹⁶ with 4,4'-diisothiocyanato-2,2'-stilbene disulphonic acid (DIDS). DIDS (added to intact cells) is found to bind almost exclusively to band 3 with less than 5% of the label associated with glycophorin.

The number of eosin molecules bound per protein is relevant to the possibility of energy transfer between eosins. For 1 µg eosin per mg total membrane protein, it may be estimated that the mole ratio eosin: band 3 would be 1:2 if all the eosin were bound to band 3 (assuming band 3 has a molecular weight of 90,000 and comprises 24% of the membrane protein).

Rotational diffusion measurements

Excitation of eosin at 540 nm by a laser light pulse of duration 1–2 µs and energy 100–200 mJ results in transient absorbance changes in the sample. Either positive absorbance changes (conveniently measured at 610–650 nm) due to triplet–triplet absorption or negative changes (measured at 500–525 nm) due to ground state depletion, may be detected. When the exciting source is plane-polarised, the absorbance changes are dichroic provided rotational motion is slower than the instrumental response (2–3 µs)^{10,11}.

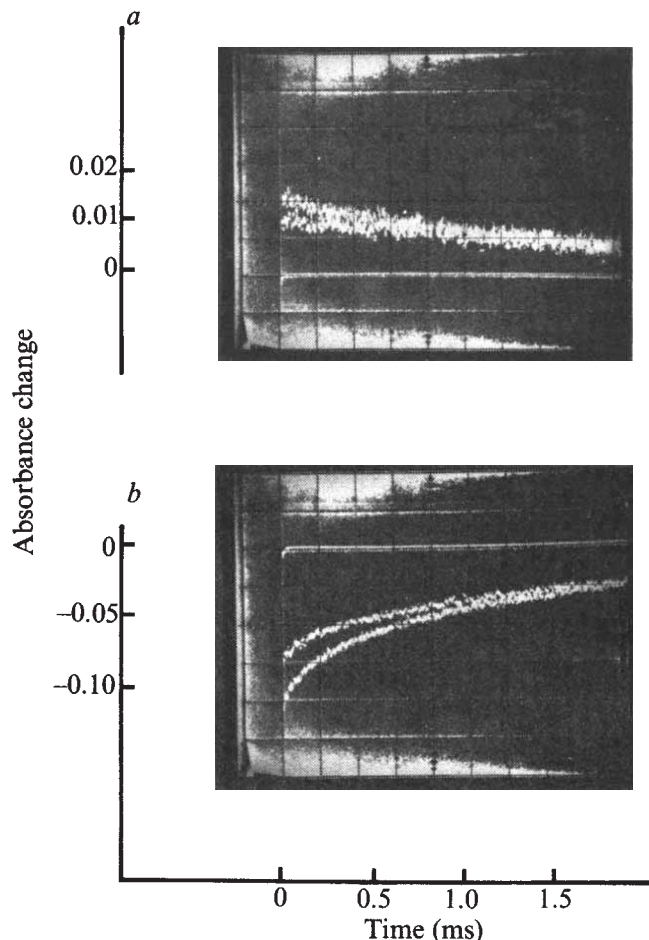
Transient absorption changes from eosin-labelled ghosts are shown in Fig. 1. The better signal-to-noise ratio of the ground state depletion signal is due to the higher extinction coefficient of the singlet–singlet transition compared with the triplet–triplet transition. Both absorption and depletion signals demonstrate the existence of a rather long lived dichroism. All quantitative evaluation of the data was carried out with depletion signals.

The data were analysed by calculating the anisotropy factor *r*(*t*) given by

$$r(t) = (A_{pa}(t) - A_{pe}(t)) / (A_{pa}(t) + 2A_{pe}(t)) \quad (1)$$

where *A*_{pa}(*t*) and *A*_{pe}(*t*) are respectively the absorbance changes at time *t* for light polarised parallel and perpendicular to the exciting flash. The time dependence of *r* is shown on a semi-

Fig. 1 Transient absorbance changes from eosin-labelled ghosts. Membranes suspended in 20 mosM phosphate buffer, pH 7.4, temperature 22 °C. Eosin concentration 7 µM, 0.9 µg eosin per mg protein. Before measurement, samples were bubbled with argon for 10 min to displace oxygen. *a*, Triplet–triplet absorption measured at 650 nm; *b*, ground state depletion measured at 525 nm. In each case the stronger signal is obtained with the measuring beam polarised parallel to the exciting flash, the weaker signal for perpendicular polarisation. The flash photolysis apparatus was described in ref. 11.



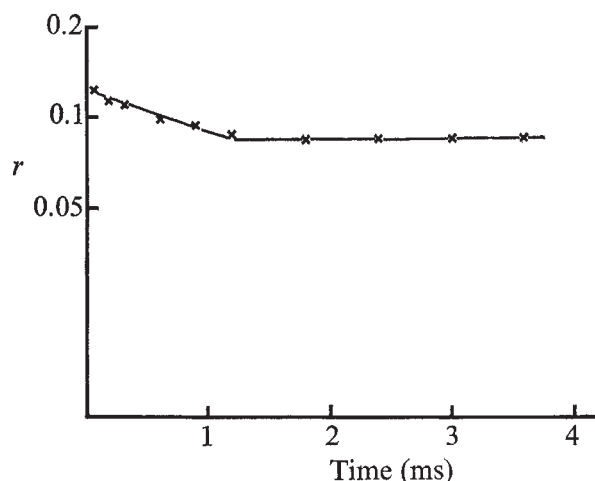


Fig. 2 Time dependence of the anisotropy parameter r calculated from depletion signals similar to those shown in Fig. 1b. For more accurate calculation the difference between the parallel and perpendicularly polarised components is displayed directly using the differential input of the oscilloscope amplifier.

logarithmic plot in Fig. 2. It is clear that the decay of dichroism contains at least two components. Within the experimental uncertainty $r(t)$ is independent of time at long times so that the observed time dependence of r takes the form

$$r(t) = r_1 \exp(-t/\varphi_1) + r_2 \quad (2)$$

Replotting $\log(r(t) - r_2)$ against t gives $\varphi_1 \approx 0.5$ ms. (The data are not sufficiently accurate to determine φ_1 precisely; conservatively, we consider the above value to be correct within a factor of 2.)

Interpretation of data

Consideration of the magnitude of the flash photolysis signals leads us to conclude that a high proportion of eosin molecules in the sample must contribute to the observed dichroism. Since we have shown that eosin is mainly bound to band 3, we can relate the dichroism data to the rotational motion of these proteins.

Equations which evaluate $r(t)$ for an irregular body in an anisotropic medium are not currently available. Various authors have, however, solved the problem of the time dependence of r for an irregular body in isotropic solution²⁸⁻³¹. If we assume that the rotational motion of band 3 proteins may be characterised by two diffusion constants, D_{pa} for rotation about an axis normal to the membrane and D_{pe} for rotation about either of the two perpendicular axes, then the problem formally becomes equivalent to the rotation of a body with an axis of symmetry immersed in an isotropic medium. We can therefore use existing theoretical treatments to evaluate our data. An additional simplification arises with depletion signals because the same transition moment is used for excitation and measurement. (The lowest absorption band of eosin is a single transition and triplet-triplet absorption is relatively small at 525 nm (refs 10 and 11).) The theoretical expression for $r(t)$ then becomes

$$r(t) = \sum_{i=1}^3 A_i \exp(-E_i t) \quad (3)$$

where $A_1 = 6/5 \sin^2 \theta \cos^2 \theta$, $A_2 = 3/10 \sin^4 \theta$, $A_3 = 1/10 (3 \cos^2 \theta - 1)^2$, $E_1 = 5D_{pe} + D_{pa}$, $E_2 = 2D_{pe} + 4D_{pa}$, $E_3 = 6D_{pe}$, and θ is the angle between the transition moment and the axis of symmetry. (The theoretical expressions given in refs 28-31 all become equivalent for the case considered here.)

We consider that there are two main interpretations of the two experimentally observed components in the decay of $r(t)$. Since band 3 proteins seem to offer different labelling sites on either side of the membrane^{12,25-27}, it is likely that they have a negligibly small rate of rotation about an axis in the plane of the membrane. The existence of a carbohydrate moiety on these proteins, which is only detectable on the outside surface, points to the same conclusion^{16,32,33}. Thus we anticipate that $D_{pe} \approx 0$ and equation (3) then gives

$$r(t) = A_1 \exp(-D_{pa}t) + A_2 \exp(-4D_{pa}t) + A_3 \quad (4)$$

Thus, the time-independent component of the experimental $r(t)$ curve may be identified with A_3 and is due to the negligibly small value of D_{pe} . ($r(t)$ will of course eventually go to zero due to rotation of the whole erythrocyte ghost; the relaxation time of this process is, however, of the order of seconds.) The measured time constant φ_1 is then related to D_{pa} ; the experimental accuracy is, however, clearly insufficient to detect the two expected components.

An alternative explanation of the data is that the eosin-binding sites are heterogeneous. (Note, however, that the amount of eosin associated with spectrin is too small to account for one or other component.) In particular, it cannot be ruled out that there is some aggregation of band 3 proteins in the membrane. In this case r_2 could correspond to aggregates which are too large to have an appreciable rotation on the time scale of the experiment.

If the first explanation is correct, we could in principle determine θ (which is likely to be a mean value), since $r_2 = A_3$. This is, however, dangerous since even if protein aggregation is not the main reason for the time-independent dichroism, it may well make a contribution. (The observation that the relative magnitudes of r_1 and r_2 vary from one preparation to another makes us suspicious that this may be the case.) We therefore prefer not to attempt to determine θ (and therefore the coefficients A_1 and A_2) and to regard φ_1 as an unknown combination

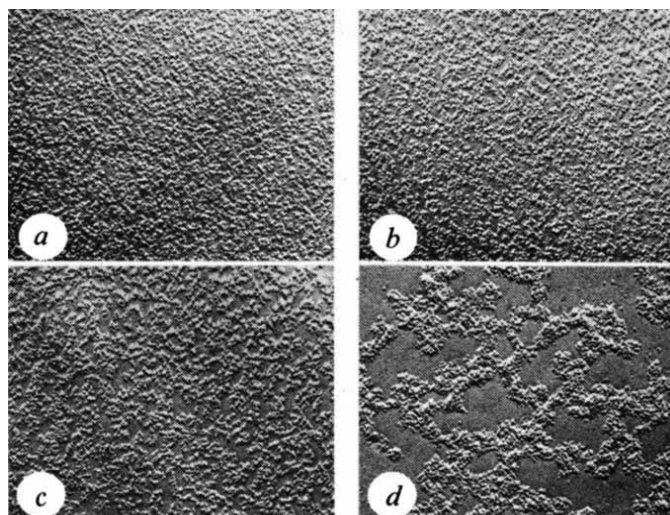


Fig. 3 Inner fracture faces (IFF) of ghosts before and after spectrin depletion ($\times 36,000$). *a*, Unlabelled, untreated, pH 7.6; *b*, eosin-labelled, untreated, pH 7.6; *c*, eosin-labelled, spectrin-depleted, pH 7.6; *d*, eosin-labelled, spectrin-depleted, pH 5.4. Eosin labelling as in text. Spectrin was depleted by incubating 1 part packed ghosts with 10 parts 20 mM phosphate buffer, pH 8.5, (containing 0.5 mM NaN_3) for 18 h at 37 °C (ref. 43). Samples were maintained at 0–4 °C at all times except during labelling and spectrin removal. For electron microscopy, samples were mounted on precooled gold disks and frozen in liquid Freon. Freeze fracturing was carried out in a Balzer's apparatus at a temperature of –100 or –105 °C. Specimens (without etching) were replicated with Pt–C and backed with C, both evaporated from electron beam guns. Replicas were examined in a Phillips 200 electron microscope.

of $1/D_{pa}$ and $1/4D_{pa}$. On this basis a value of 0.5 ms for ϕ_1 implies D_{pa} is in the range 500–2,000 s^{-1} .

Band 3 is probably involved in anion transport across the erythrocyte membrane^{15,16,34}. Lepke *et al.*³⁵ find that sulphate exchange is completely inhibited when 1.7×10^6 molecules of the irreversible inhibitor dihydro 4,4'-diisothiocyanato-1,2-diphenyl-ethane-2,2'-disulphonic acid (H_2DIDS) are bound to band 3. This figure implies a turnover number for chloride exchange of about 2×10^4 per s per site if all these sites are involved in transport. If only a portion of these sites are responsible for transport then the turnover number must be higher. Thus a rotation time in the order of tens of microseconds or faster would be required if transport occurred by a rotational mechanism. Irrespective of any particular interpretation, the present experiments do not detect any component of rotational motion of band 3 in this order of magnitude. Thus if band 3 is responsible for anion transport, a mechanism involving rotation of the whole protein is ruled out. This is hardly a surprising conclusion; as discussed above, arguments based on the structure of band 3 also indicate that rotation across the membrane is improbable^{16,25}. Nevertheless, the present studies demonstrate how rotation measurements may be useful in elucidating transport mechanisms in other systems where the outcome is not so easily anticipated.

'Viscosity' of the membrane

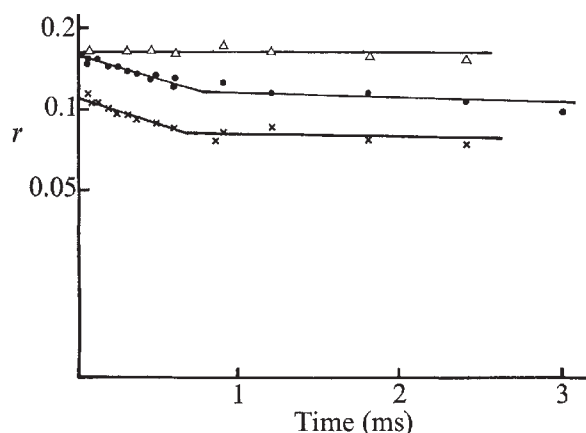
Whatever the precise interpretation of the data, a component of rotational motion of band 3 proteins of the order of 0.5 ms is clearly present. The only existing quantitative determination of the rotation of a membrane protein is Cone's measurement with rhodopsin², which yielded a relaxation time of 20 μs at 20 °C. Rotation of band 3 proteins in the human erythrocyte membrane clearly is markedly slower.

We found that addition of glycerol to a concentration of 60% to the membrane suspension had no detectable effect on ϕ_1 . Since this addition increased the medium viscosity by a factor of 10, we may conclude that the rotation of band 3 proteins is a function of the membrane viscosity. If we regard band 3 proteins as cylinders of radius a , then the friction coefficient for rotation about the axis of symmetry is given by (ref. 36)

$$f = 4\pi a^2 h \eta \quad (5)$$

where h is the depth of the cylinder immersed in the membrane and η is the membrane viscosity.

Fig. 4 Effect of spectrin removal on the time dependence of the anisotropy parameter r . Temperature 22 °C. $\times$, Control sample, pH 7.6; $\bullet$, spectrin-depleted ghosts (prepared as described by Fairbanks *et al.*²²), pH 7.6; $\triangle$, spectrin-depleted ghosts, pH 5.4. The lower initial value of r for the control sample is due to experimental factors and has no special significance. Essentially similar results were obtained when spectrin was depleted using the procedure of Elgsaeter and Branton⁴³.



From the Einstein relation³⁷

$$D = kt/f \quad (6)$$

we obtain

$$D_{pa} = kt/4\pi a^2 h \eta \quad (7)$$

If we take a as the radius of the particles revealed by freeze-fracture electron microscopy (40 Å) and h as the thickness of the hydrophobic core of the membrane (40 Å), then a value of $\phi_1 = 0.5$ ms implies $\eta = 25$ –100P (remembering that the respective contributions of $1/D_{pa}$ and $1/4D_{pa}$ to ϕ_1 have not been determined). This is considerably higher than previous estimates of the viscosity of the erythrocyte membrane based on measurements of methanol diffusion³⁸ and fluorescence polarisation^{39,40}. It may indicate that the 'microviscosity' experienced by small molecules dissolved in the hydrocarbon region of the lipid bilayer is quite different from that experienced by a protein which may interact with the whole length of the lipid molecules.

Probably the replication procedure used in freeze-fracture electron microscopy leads to an overestimate in the size of the particles^{41,42}. If the true radius of band 3 proteins is less than 40 Å, then the calculated viscosity will be increased to still higher values.

Interaction between band 3 and spectrin

The above discussion of membrane viscosity only has validity if band 3 proteins undergo free diffusion in the membrane. Apart from the possibility of self-association of these proteins discussed above, possible interactions with other components of the membrane have also to be considered. In particular, there is evidence that spectrin restricts lateral diffusion of intramembrane particles⁴³. It has been proposed that these particles contain both glycophorin and band 3 and are physically linked to spectrin¹⁹.

We have investigated the effects of spectrin removal on rotational diffusion of band 3 proteins. We have depleted eosin-labelled ghosts of spectrin using the procedure of incubation at low ionic strength described by Elgsaeter and Branton⁴³. Figure 3 shows freeze-fracture electron micrographs of unlabelled ghosts, eosin-labelled ghosts and spectrin-depleted ghosts, all at pH 7.6, together with spectrin-depleted ghosts at pH 5.4. The distribution of particles is little different in the labelled ghosts compared with the control, demonstrating that the labelling procedure in itself does not cause any marked aggregation. In agreement with Elgsaeter and Branton⁴³, the distribution of particles in spectrin-depleted ghosts is little altered at pH 7.6 but massive aggregation occurs on lowering the pH to 5.4.

Spectrin is most efficiently and selectively removed by incubation at low ionic strength in the presence of EDTA. Using the procedure of Fairbanks *et al.*²² we have also prepared spectrin-depleted ghosts in this manner. SDS-polyacrylamide gels confirmed that most of bands 1, 2 and 5 were selectively released from the membrane. When examined by electron microscopy it was clear that considerable vesiculation of the membranes had occurred, as noted by other authors⁴⁴. The effects of pH on particle aggregation was, at far as could be ascertained, essentially similar to that shown in Fig. 3.

Spectrin-depleted ghosts prepared by both methods outlined above were examined by flash photolysis. Essentially similar results were obtained in both cases; one set of data is shown in Fig. 4.

Inspection of Fig. 4 clearly shows that there is no detectable rotational motion in spectrin-depleted ghosts at pH 5.4. This is entirely reasonable in view of the strong aggregation of particles in these conditions as seen in Fig. 3. Large aggregates would be expected to have negligible rotation on the time scale of the experiment. This correlation between rotational motion and the state of aggregation of the particles further demonstrates that we are making valid measurements of rotational motion of band 3 proteins (or alternatively, provides further evidence that the particles contain band 3 proteins).

The most interesting conclusion which arises from the results in Fig. 4 is that there is no detectable difference in rotational motion between spectrin-depleted ghosts and control ghosts when the pH is maintained at 7.6. In other words, there is no evidence that spectrin restricts the rotational diffusion of band 3 proteins. Although this finding does not completely rule out a direct linkage between band 3 and spectrin, it does place severe restrictions on the type of attachment which can be envisaged. Only a linkage which permits free rotation of band 3 about an axis normal to the plane of the membrane is compatible with the present measurements.

On the other hand, we consider that a model in which there is no direct interaction between band 3 and spectrin accounts equally well for all the mobility data. Suppose that band 3 proteins protrude from the membrane in the spaces between the spectrin fibres. It follows that lateral diffusion over large distances would be prevented by the spectrin network, whereas rotational diffusion would be unaffected. The enhanced aggregation of membrane particles in spectrin-depleted ghosts at low pH (ref. 43) would be explained by such a model. Further, proteins entrapped within the spectrin network would probably follow any redistribution of the spectrin molecules themselves. The clustering of anion sites⁴⁵ and concanavalin A receptors¹⁹ on the outer membrane surface which is observed when spectrin is aggregated may be explained in this way.

Peters *et al.*⁴⁶ labelled proteins (principally band 3) with fluorescein, bleached one half of a single ghost and looked for the spread of fluorescence from the unbleached to the bleached half. No spreading was detected, placing an upper limit on the lateral diffusion constant of $3 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$. For free diffusion such a low value would imply a rotational relaxation time of the order of 50 ms, a value clearly incompatible with our 0.5 ms component of rotational motion. This again implies that lateral diffusion is restricted and is entirely compatible with a model of band 3 proteins trapped in the spectrin net.

Our results emphasise the importance of clearly distinguishing local diffusion from diffusion over large distances. The fact that the latter is not observed does not necessarily imply that proteins are immobilised. If the membrane is 'compartmentalised' then rapid diffusion over small distances may still occur and in turn may have functional significance.

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letters to nature

Optical behaviour of HDE226868 during a Cyg X-1 X-ray transition

As part of regular monitoring of variable X-ray sources with the University College London-Mullard Space Science Laboratory X-ray instrumentation on the Copernicus satellite, we have observed Cyg X-1 continuously for 12 d between 22 October and 3 November, 1975. The X-ray flux was noted to be more variable than it had been during previous monitoring runs, when the source had been in its low state¹, and, in particular, a marked increase in flux occurred 8 d into the observing run (~ October 29.5 UT, 1975). After this date, the mean 3-8-keV flux increased steadily, and by 3 November had risen by 30% compared with its level during the first part of the observation. The X-ray flux data, integrated in time blocks of 30 min, are shown in Fig. 1b. In Fig. 1a we show the spectral hardness ratio (defined as the 5.5-8.0-keV flux divided by the 3.0-5.5-keV flux) integrated over intervals of 0.5 d. This quantity decreased

progressively after 30 October, until by 3 November it was down by an amount equivalent to a drop of 0.1 in the power law spectral index of the source.

Holt *et al.*² and Grindlay *et al.*³ have also noted an increase in the X-ray flux of Cyg X-1 at about this time. These two groups found the sources to be up to five times stronger than the

Table 1 Best-fit parameters and χ^2

Interval	χ^2 per degree of freedom	Mean (m_B)	Semi-amplitude (m_B)
A	23.9	9.644	0.025
B	18.6	9.660	0.018
C	28.4	9.661	0.022
D	32.5	9.650	0.018
E	18.8	9.649	0.016
F	7.7	9.640	0.028
G	19.1	9.650	0.020
All 1975 data	22.0	9.652	0.020

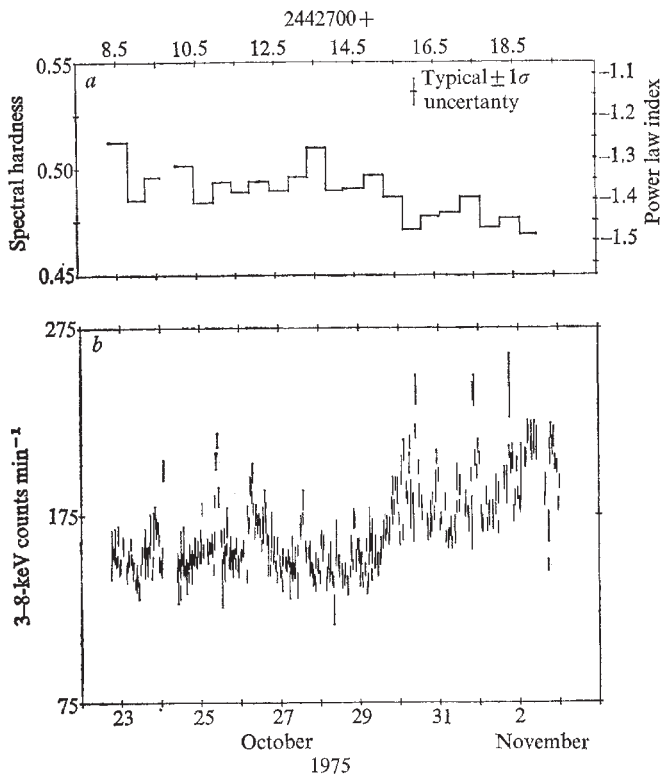


Fig. 1 Copernicus X-ray data for Cyg X-1 during October–November 1975. *b*, X-ray flux binned into 30-min intervals; *a*, spectral hardness ratio in 0.5-d bins. The error bars correspond to $\pm 1\sigma$ in each plot and there is a systematic uncertainty of up to 0.1 in the power law index indicated on the right-hand ordinate of *b*.

level seen earlier in October, a maximum equivalent to $\approx 1,500$ Uhuru counts being seen on 6 November³. Continued observation with the Ariel V satellite^{4,5} has shown that Cyg X-1 maintained a relatively high flux level until late February, when the intensity dropped by a factor of three⁵.

The blue-band optical magnitude of HDE226868, the spectroscopic binary BO1b star which is the optical counterpart of Cyg X-1, was monitored between July and December 1975 from the Spanish Sierra Nevada. The data are shown in Fig. 2, and are compared with the X-ray behaviour of Cyg X-1 as measured by Copernicus and inferred from the Ariel V and ANS data reported in refs 2 and 3. The crosses represent nightly mean blue magnitudes and have a typical standard error of $\pm 0.003m_B$.

We note, first, that the mean B-band flux reaches a local maximum close to the time of the X-ray transition. This may be coincidental, however, since such maxima are not unprecedented and have not previously been correlated with any known X-ray event. Of more significance is the detailed form of the blue light curve about the time of the X-ray transition. As observed in previous years, the average blue light curve

for the 1975 data is typified by a distorted double sine wave per orbital period^{6–8}. To quantify the light curve distortion, we have fitted a double sine wave to the individual three-orbit intervals marked in Fig. 2, allowing the amplitude and mean magnitude to vary so as to minimise χ^2 . The phase of the double sine wave was taken to be the mean phase obtained by fitting all of the 1975 data.

The best-fit parameters and the corresponding reduced χ^2 for each interval are shown in Table 1 and light curves for the intervals about the time of the X-ray transition are shown in Fig. 3. The data points and the best-fit double sine wave for interval E are shown in Fig. 3*a*. This light curve is typical of the data from July to October 1975, and the large values of χ^2 for intervals A to E indicate the relatively large departures from a double-sine-wave light curve.

A change in the character of the light curve was observed, starting on the night of 25/26 October, as shown in Fig. 3*b*. On this night the B-band flux was abnormally faint, and then increased to an abnormally high value two nights later. No light variations were then seen over the following three nights.

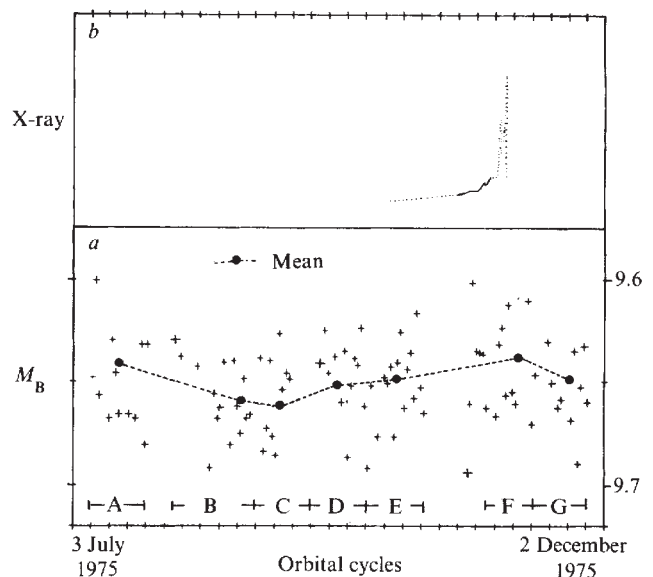


Fig. 2 *a*, Blue-band magnitude of HDE226868 from July 1975. The filled circles represent means (uncertainty $\sim 0.004m_B$) over the intervals marked (~ 3 orbits). The X-ray flux from the present work (—) and from refs 3 and 4 (---) are shown in *b*. Times of superior conjunction of the X-ray source are indicated on the abscissae.

During the interval immediately after the X-ray turn-on, regular light variations were again observed, as indicated in Fig. 3*c*, which shows data and the best-fit double sine wave for interval F. The degree of departure from the best-fit curve was, however, considerably reduced, as demonstrated by the low value of χ^2 for this interval. (Note that the six data points shown in Fig. 3*b* are not included in interval F.) Distortion of

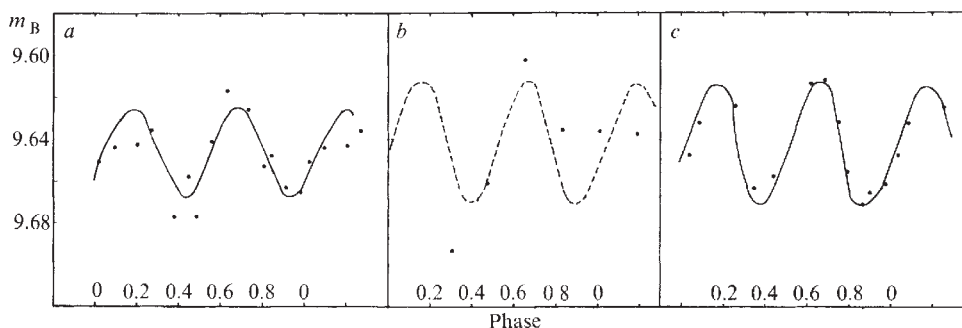


Fig. 3 *a* and *c*, Blue-band data folded with the 5.6-d orbital period. *b*, Data (unfolded) for the 6 d spanning the transition in X-ray flux noted in the text. Formal errors of measurement are $\pm 0.003m_B$. The best-fit double sine curves are indicated for the data in *a* and *c*. The best-fit curve to the data shown in *c* is superimposed on the unfolded data in *b*, for reference. *a* (Interval E), JD 2442684–698; *b*, JD 2442711–716; *c* (Interval F), JD 2442717–736.

the light curve became more pronounced again towards the end of the 1975 data, as indicated by the higher value of χ^2 for interval G. It is important to note that the behaviour described above is unprecedented in a total of 400 nights of optical monitoring of the source distributed through the past 4 yr.

As the unique change in the optical behaviour of HDE226868 coincides to within one orbital period with the onset of the transition of Cyg X-1 to its high state, it is highly probable that the X-ray and optical events are related. Our immediate conclusion is that the optical variability is influenced by the condition of the X-ray source, and if ellipsoidal-like variations, as discussed by Hutchings⁹ and Avni and Bahcall¹⁰, do occur, then at times they are insignificant by comparison with this other mechanism.

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Suspected globular clusters in the Fornax I cluster of galaxies

HERE we report the existence of a significant clustering of faint objects around three galaxies in the Fornax I cluster of galaxies. The nature of these objects is uncertain, although on the scant evidence available, we believe at least some of them to be unresolved globular clusters. We base our claim on the examination of a single, direct photographic plate taken by Dr R. D. Cannon at the prime focus of the Anglo-Australian telescope on the night of January 18, 1975. The plate, AAT609, is an unsensitised IIaO, exposed for 20 min through a GG 385 filter, and covers an area of $1^\circ \times 1^\circ$ centred on RA 3 h 36 min, dec. -35.5° (1950.0). The smallest images on the plate are slightly elliptical, owing to slight trailing in RA, and have dimensions $1.5'' \times 2.0''$. In the course of assigning morphological types to the brightest galaxies in the field, we found a noticeable increase in the number of faint objects in the vicinity of the galaxies NGC1374, NGC1379 and NGC1399. These three galaxies are all elliptical and are among the nine brightest on the plate. Of the remaining six bright galaxies, two (NGC1389 and NGC1404) are elliptical and four (NGC1375, NGC1380, NGC1381 and NGC1387) are lenticular; none of these six galaxies exhibits any significant enhancement in the number of adjacent field objects.

In Fig. 1, we show the marginal distributions in RA and dec. of the faint objects around the three galaxies, and in

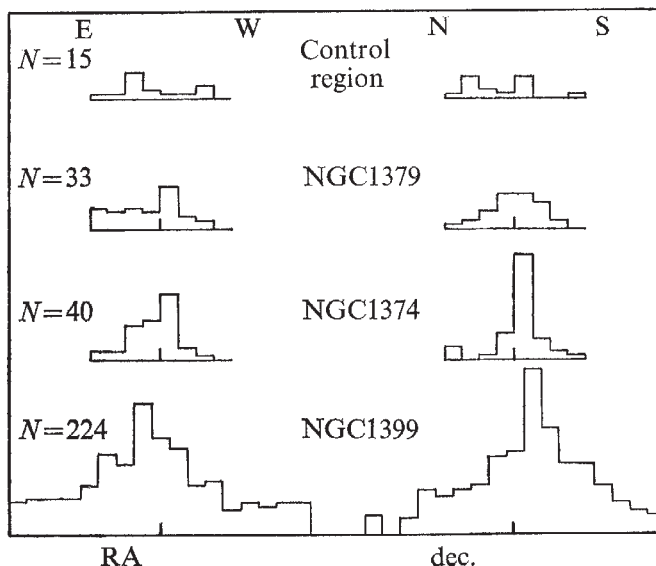


Fig. 1 Marginal distributions of faint objects around three galaxies in Fornax I, and in a control region. The ordinates are in the same arbitrary units for all the histograms. N represents the total number of faint objects in each area measured. The widths of the histograms for NGC1399 are both $10.7'$, those for the remaining regions are $5.0'$.

a control area centred on a star at RA 3 h 34 min, dec. -35.3° , well removed from any bright galaxy. The counts were made visually with the aid of a low-powered microscope and a rectangular grid (of unit size $18.9'' \times 18.9''$ on the plate), and the positions of all objects were recorded. The area measured round NGC1399, the brightest of the three galaxies, was $10.7' \times 10.7'$, whereas the corresponding areas for the other two galaxies and the control region were $5.0' \times 5.0'$. Those objects judged to be faint were selected solely on the criterion of image size and not of image structure. They were estimated to be within 1 mag of the limiting magnitude of the plate. In all, 224 were found around NGC1399, 40 around NGC1374 and 33 around NGC1379. For comparison, the number of faint objects in the control region was 15.

The density distribution of faint objects around NGC1399, derived from ring counts (Fig. 2) has been fitted with a truncated isothermal distribution assuming spherical symmetry. The asymptotic value of the density of $3.4 \times 10^3 \pm 1.4 \times 10^3$ (s.e.) per degree² is slightly higher than the density of 2.2×10^3 per degree² found in the control region. In view of the differences in the dispersion of the marginal distributions in RA and dec. (Fig. 1), we thought it necessary to check the assumption of spherical symmetry. We have fitted the observations with a bivariate Gaussian distribution and found major and minor axes of $154''$ and $133''$ (that is, an ellipticity of 0.13), and that the position angle of the major axis is 105° . This weakly confirms the impression given to the eye that the cluster of faint objects around NGC1399 is slightly elongated in the direction of the major axis of the galaxy (position angle 115°). The centroid of the distribution however, lies $28''$ to the SE of the centre of NGC1399, and there is a relative paucity of objects in the NE sector of the galaxy. The NGC1374 system seems symmetrical and well defined. The smaller number of objects around NGC1379 inhibits any assessment of the symmetry of the distribution.

The average density of all stellar images on the plate is 4×10^3 deg⁻². If we tentatively identify these with foreground stars within our Galaxy, then we may extrapolate an approximate value for the limiting magnitude of the plate from tables of star numbers given by Allen¹ (with the appropriate correction for magnitude scale errors given by

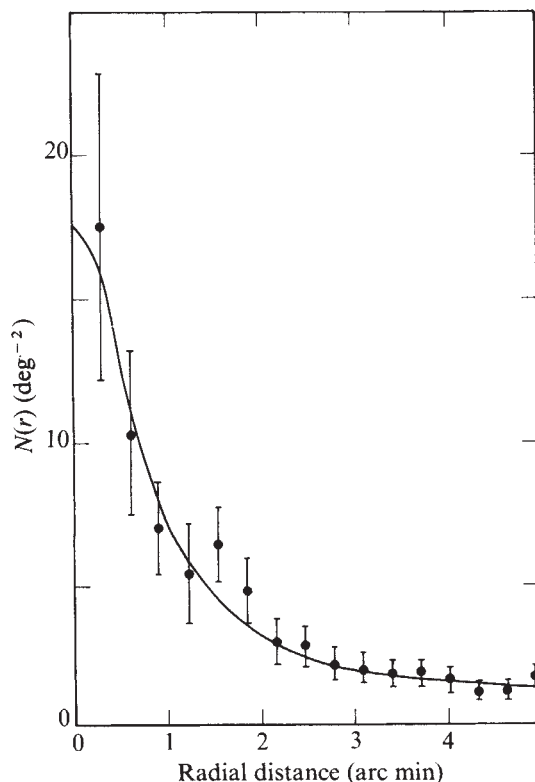


Fig. 2 The radial density distribution (number arc min⁻²) of the faint objects around NGC1399 (dots, with corresponding error bars). The best isothermal fit is shown by the continuous curve.

Arp³). For the galactic latitude of Fornax I, $b^{\text{II}} \sim -54^\circ$, the above density corresponds to a limiting B magnitude of 22.5 ± 1.5 mag (estimated error). Although plate AAT609 was not calibrated, another estimate of its limiting magnitude may be obtained from a comparison with plate AAT610 taken on the same night. This is a B plate of NGC288, which contains a standard photoelectric sequence (R. D. Cannon, personal communication), and has a limiting magnitude of 21.6 ± 0.5 mag ($\pm$ s.e.). Allowing for slight differences in exposure times ($\Delta m = -0.4$ mag), air masses ($\Delta m = +0.2$ mag) and seeing ($\Delta m = +0.2$ mag), we conclude that an appropriate average value for the limiting magnitude of AAT609 is 22.0 ± 0.5 mag ($\pm$ s.e.).

The corrected redshift of Fornax I (ref. 3) is $z = 0.0050 \pm 0.0003$ ($\pm$ s.e.) and, if $H = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$, this corresponds to a provisional distance of 27 Mpc. If the faintest of the suspect objects is characterised by an angular size of less than $2''$ and an apparent B magnitude of 22.0 mag, then at this distance these parameters correspond to a linear dimension of less than 260 pc and an absolute B magnitude of about -10 mag. We suggest that these objects are unresolved globular clusters, although there are some difficulties associated with this hypothesis, which we discuss below.

The existence of globular clusters around a number of galaxies in the Virgo cluster^{4,5} and, in particular, around the active elliptical M87 invites a comparison. Racine⁴ has analysed the distribution of 2,000 globular clusters around M87 and more recently Hanes⁶ has studied globulars associated with twenty galaxies (including M87) in the Virgo cluster. Hanes (personal communication) has revised Racine's conclusions regarding the luminosity function of the M87 globulars. He finds that there is no sharp cutoff at the bright end of the luminosity function, but rather a Gaussian tail with the brightest globular occurring at about $B = 20.4$ mag. The brightest of the Fornax objects lies at about 1 mag above the plate limit and has an apparent

B magnitude of 21.0 ± 0.6 mag. The hypothesis that the upper limit to globular luminosities, around any given galaxy, is set by statistical fluctuations in random samples of various sizes, has been used by de Vaucouleurs⁷ to substantiate an empirical linear correlation between the absolute magnitude of the first-ranked globular and the total absolute magnitude of the associated galaxy. We have used this correlation to obtain corrected distance moduli for M87 and NGC1399 of 30.4 mag and 30.8 mag respectively (allowing for 0.2 mag of extinction in both cases) and absolute magnitudes of the two galaxies of -21.1 mag and -20.2 mag for the corrected B magnitudes of 9.35 mag and 10.62 mag (ref. 8). The corresponding absolute B magnitudes of the brightest globulars in the two systems are at -10.0 mag and -9.8 mag. M87 has, however, been suspected to be abnormally rich in globular clusters (D. A. Hanes, personal communication) and a more detailed calculation will be presented later (de Vaucouleurs and J. A. D., unpublished). The difference in the distance moduli of M87 and NGC1399 is 0.4 mag, which corresponds to a distance ratio of 1.20. This may be compared with the ratio of the corrected redshifts³ for the E cores of Fornax I and Virgo, which equals 1.45.

Fornax I lies within the Southern Supercluster which stretches from Cetus to Dorado⁹. The background density of faint galaxies is high and non-uniform, and the possibility that the clusters of faint objects around the three galaxies might result from chance alignments must be considered. This possibility is supported by the observation that there are few faint objects around NGC1404, even though it is a galaxy of similar luminosity to NGC1399. Although the system around NGC1399 is the most populous and extensive, the marked asymmetry of the distribution about the galaxy must raise doubts as to whether the two are physically related. Indeed, a Coma-like cluster of galaxies, removed to forty times the distance of the Coma cluster, would match the distribution both in angular extent and apparent magnitude of the 10th-ranked member. The number of objects around NGC1379 is sufficiently small to be accounted for as a local enhancement in the number of field objects, but in NGC1374 there is a system which gives the firm impression of a symmetrically placed distribution of globular clusters.

Smith and Weedman¹⁰ have reported the discovery of globular clusters around NGC3311 in the Hydra I cluster of galaxies and, in common with others^{4,5,7}, have discussed their use as distance indicators leading to an evaluation of the Hubble constant. Fornax I is about 150° away from the supergalactic centre in Virgo and is well placed to reflect any apparent anisotropy in the Hubble flow which may arise from systematic motions within the local supercluster.

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Night-time reception of a solar radio event

ON March 30, 1976, at 2104–2106 UT, anomalous high-level radio bursts were recorded with a 48-channel polarised high resolution radio spectrograph¹ operating at a frequency range of 20.85–23.20 MHz. The observation was made at Kiiminki (65°05'N, 25°54'E), in the complete absence of interference of any kind and with the Sun and Jupiter well below the horizon. The antenna (two crossed log-periodic units in parallel) was directed towards North and elevated 30° for the purpose of recording scintillation spectra of the radio source Cassiopeia A. The spectrograph is designed primarily for observations of Jupiter² and its sensitivity for recording Cassiopeia A is marginal.

The anomalous bursts are shown in Fig. 1. The power level of the bursts exceeds the highest scintillation peaks of Cassiopeia A by 10–15 dB. The level is comparable to

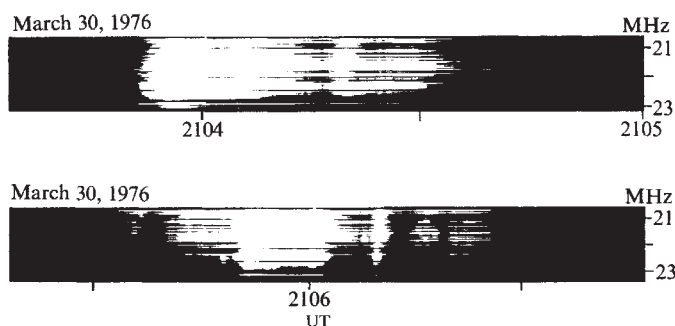


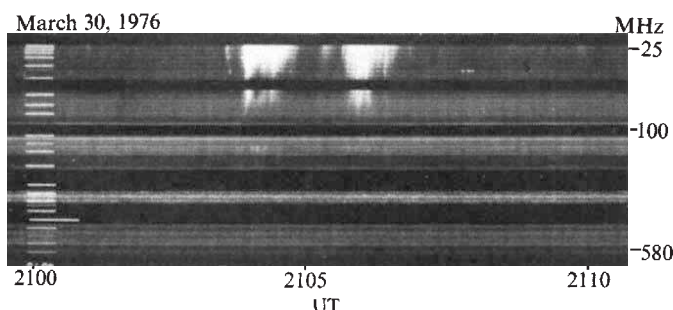
Fig. 1 Dynamic spectra of anomalous night-time radio bursts.

that of the strongest Jovian bursts observed close to the opposition of the planet. The colour^{1,2} of the original record indicates that the right- and left-circular components of the emission had equal amplitudes.

At the same time, a strong solar radio event was in progress. From the radio spectra obtained at the CSIRO Solar Observatory, Culgoora, Australia; at the Harvard Radio Astronomy Station, Fort Davis, Texas (see Fig. 2); and at the Department of Astro-Geophysics, University of Colorado, Boulder, Colorado, it is inferred that the bursts are of solar type III. As can be seen from Fig. 2, the bursts start in the vicinity of 80 MHz. At the lowest recorded frequency (25 MHz) they match well with the bursts shown in Fig. 1.

Anomalous propagation of solar bursts have been observed previously³. In the present case the bursts must have been guided from the sunlit hemisphere over the polar regions. It may be significant that on the night of March 30, an auroral substorm and a strong blanketing sporadic-E (E_s) were observed (ref. 4 and T. Turunen, personal communication). A suitable duct may have existed between the ground and the E_s layer. This assumption is supported by

Fig. 2 The corresponding record of solar radio bursts, obtained at Fort Davis, Texas.



the low attenuation of the bursts. From their estimated flux density (A. Maxwell, personal communication) it is deduced that the total attenuation was less than 10 dB, probably just a few dB. The waves first penetrated the F2 layer, perhaps at a relatively high latitude, and then entered the duct beneath the E_s layer, provided the latter extended far enough towards the sunlit hemisphere.

The event was also recorded with a 20-MHz riometer at the Sodankylä Geophysical Observatory (67°25'N, 26°24'E). It appears as an outstanding peak signifying an increase in the noise level. It was not detected with the 27.6-MHz riometers at Sodankylä or at stations elsewhere in Finland (H. Ranta, personal communication) although strong solar emission occurred also at this frequency.

I thank Drs A. Maxwell, G. A. Dulk, and K. V. Sheridan for the Fort Davis, Boulder, and Culgoora solar records, and Mrs H. Ranta and Mr T. Turunen for the Sodankylä riometer and ionospheric data.

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Absorption and the low velocity zone

IT is well known that elastic wave velocities are independent of frequency only for a non-dissipative medium¹. In a real solid dispersion must accompany absorption. This effect has been emphasised by Randall² and Liu *et al.*³. The effect is small when the seismic quality factor Q is large or unimportant if only a small range of frequencies is being considered—that is, the spectra of P waves. Even in these cases, however, the measured velocities, or inferred elastic constants, are not the true elastic properties but lie between the high frequency and low frequency limits or the so-called 'unrelaxed' and 'relaxed' moduli. The magnitude of the effect depends on the nature of the absorption band and the value of Q . When comparing data taken over a wide frequency band the effect of absorption can be considerable especially considering the accuracy of present body-wave and free-oscillation data. Thus, Jeffreys⁴ questions conclusions based on free oscillation data, particularly where they differ from body-wave results which are based on much shorter periods. Carpenter and Davies⁵ and Davies⁶ attempted to reconcile body-wave and surface-wave Earth models by allowing for physical dispersion using Futterman's⁷ and Kolsky's⁸ dispersion-absorption relationships. Jeffreys⁴ used Lomnitz's⁹ relationships. Liu *et al.*³ and Anderson *et al.*¹⁰ have shown how to correct surface-wave and free-oscillation data for physical dispersion. Much of the support for the existence of an upper mantle low velocity zone has come from the inversion of normal mode data that have been uncorrected for physical dispersion due to absorption. In the light of these developments, we decided to re-examine the question of a shear-wave low velocity zone.

A starting model was constructed with monotonically increasing velocity and density in the upper 400 km of the mantle. The P and S velocities were chosen to satisfy the Jeffreys–Bullen¹¹ travel time tables to 30°. A smooth, Bullen¹² model A, density structure was used for this region. The model has a 3-km thick ocean layer, and an 18-km thick crustal layer.

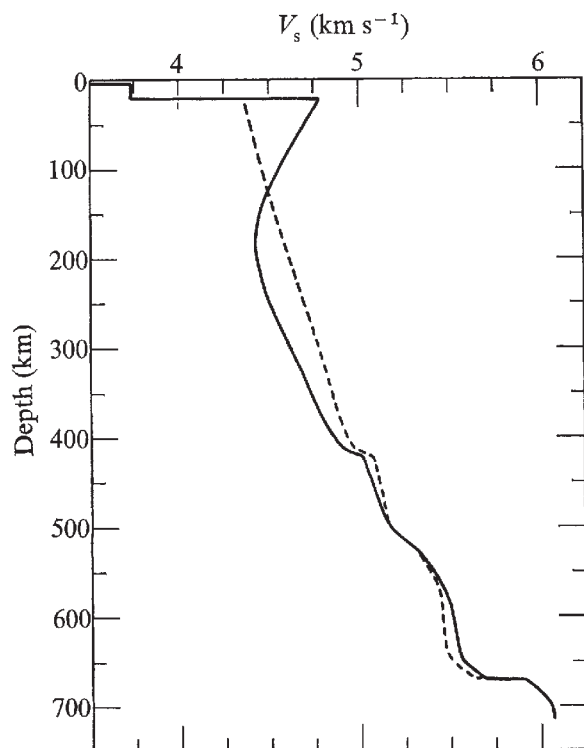


Fig 1 Shear velocity (V_s) as a function of depth for the starting model (dashed) and final model (solid).

These are average values for the Earth¹³. Below 400 km the parameters are the same as model C2 of Anderson and Hart¹³.

Following Anderson *et al.*¹⁰, the observed eigenperiods were corrected for the effect of absorption. A reference period of 1 s was selected to enable comparison with body-wave results. The corrected data included 66 fundamental spheroidal modes, 46 fundamental toroidal modes, 10 spheroidal overtones and 80 toroidal overtones. The spheroidal modes were selected for their sensitivity to shear velocity. The Q model MM8 of Anderson *et al.*¹⁴ was used to compute the correction. This data set was then inverted, following the technique described by Jordan and Anderson¹⁵. The shortest smooth perturbation from the starting model which satisfies the data is found by this procedure.

The starting model does not provide a satisfactory fit to the normal mode data. The r.m.s. error is 0.7% whereas the r.m.s. error of the data is 0.09%. Only three modes are fit to within 1 s.d. of the data, and the errors for the shorter fundamental spheroidal modes (Rayleigh waves) are as large as 1.9%. The final model fits the data with an r.m.s. error of 0.07%; of the representative 78 modes used in the inversion, 62% fit the data to 1σ and 95% fit to 2σ .

The resulting upper mantle shear velocity (solid line) is shown in Fig. 1, together with the starting model. The new model has a pronounced low velocity zone with shear velocity decreasing from 4.77 km s⁻¹ at the top of the mantle to a minimum of 4.42 km s⁻¹ at 170 km. The average JB shear-wave travel time residual for the new model is ± 0.05 s over the distance range 30–95°. This can be compared with the 4–6-s discrepancy found from previous studies in which absorption was ignored^{13,15}.

We agree with Jeffreys⁴, Davies⁶, and Carpenter and Davies⁵ that absorption must be taken into account when inverting surface-wave and free-oscillation data. Although some important revisions are required to Earth models determined from previous studies^{13,15} we conclude that a low velocity zone is required by the observed data. There is no longer any 'baseline' discrepancy between body waves and normal modes. A more complete inversion which utilises the complete spheroidal mode data set will be reported elsewhere.

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Effect of nearby supernova explosions on atmospheric ozone

We have calculated the probable effects of a nearby supernova event on the ozone layer. We find that the effects are significant and long lasting, but are relatively rare at the location of the Earth in the Galaxy.

Ruderman¹ has suggested that cosmic-ray particles and γ rays produced by a nearby supernova could cause a substantial depletion of the Earth's ozone shield, with a consequently strong increase in the solar ultraviolet radiation incident on the surface of the Earth.

The suggested mechanism for ozone destruction is the formation by incident ionising radiation of large quantities of nitrogen oxide (NO_x) in the stratosphere. The NO_x then catalytically converts the ozone (and atomic oxygen) to molecular oxygen through the reaction sequence



Ruderman has estimated the reduction in ozone column density that would result from a 10^{47} – 10^{48} -erg burst of γ rays with a duration similar to that of a visible supernova event, that is, short compared with the residence time of NO_x in the stratosphere. He has also estimated the reduction that would result from an extended 'pulse' of cosmic rays with a total energy of $\sim 10^{50}$ – 10^{51} erg and a duration² of ~ 100 yr, the characteristic diffusion time over the distance from the supernova. The reductions obtained are quite dramatic; up to 90% in the case of the γ -ray burst.

We have applied more recent astronomical observations and somewhat different astrophysical concepts to the calculation of the intensity of ionising radiation expected from a supernova event. More refined models³ of atmospheric chemistry and transport have been used to calculate the ozone depletion attributable to NO_x catalysis. We conclude that the ozone depletion, although smaller than that estimated by Ruderman, is still significant, and could, as a result of cosmic rays, extend over a very long period of time (10^3 – 10^4 yr); however, the probability of such an occurrence within the past 10^8 yr seems to be low.

In calculating the effect of γ rays, we first considered the possibility, as did Ruderman, that observed pulses of γ rays

in the 1–500-keV range⁴ are attributable to extragalactic supernovae. This gives an upper limit on the energy content of a supernova γ -ray pulse of 10^{47} – 10^{48} erg. Following Ruderman, we assume that all of the incident energy deposited at an altitude of 25–35 km. Our model enables us to follow the dynamics of the response of the atmosphere to a pulse, which we took to be a square wave of duration equal to the duration of the optical event (~ 100 d). Our calculations show that the occurrence of such an event 10 pc from Earth would result in peak ozone-column reduction of 35–65% for the 10^{47} and 10^{48} erg (upper limit) pulses, respectively. Such values are smaller than, but comparable to, Ruderman's estimates. As the e -folding recovery time of the stratosphere (the stratospheric residence time of NO_x) is ~ 3 yr, the time for the atmosphere to recover to 95% of normal would be ~ 10 yr.

Direct observations^{6,8} of supernovae before, during, and after optical maximum give an upper limit to the emitted power in the 1–500-keV range of 10^{41} erg s^{-1} , which is compatible with the 10^{47} – 10^{48} erg assumption discussed previously. Recent searches for smaller bursts⁷ seem, however, to favour the hypothesis of galactic origin, reducing the estimated intrinsic burst energy by a factor $\sim 10^6$. Such an energy would be completely insignificant with respect to ozone reduction.

In previous calculations of the terrestrial effects of the cosmic rays produced by nearby supernova events, such as those by Ruderman¹ or by Laster², it has been assumed that the explosion accelerates a substantial fraction of the envelope to relativistic velocities, producing a 'pulse' of cosmic rays of energy comparable in magnitude to the total energy of the event (10^{50} – 10^{51} erg). Recent spectroscopic observations suggest, however, that this is not the case⁸, and tend to support models of the outburst which do not predict cosmic-ray 'pulses' of any significant energy⁹. It is known, however, that supernova remnants (SNR) expanding into the interstellar medium contain trapped cosmic-ray particles of high energy. The total energy content of SNRs can be deduced from the observed synchrotron radiation from trapped electrons. Our approach, then, is to assess the effects of envelopment of a planet in an expanding SNR containing trapped cosmic rays. The effects of such radiation would persist for the lifetime of the SNR ($\sim 10^3$ – 10^4 yr) and would be expected to be more biologically significant than a 10 – 10^2 -yr effect of comparable magnitude.

To estimate the total energy contained in the form of trapped cosmic rays, W_{cr} , we have taken values of W_{cr} given by Ginsburg and Syrovatskii¹⁰ for eight remnants with radii between 2 and 20 pc, and corrected them using more recently determined radii¹¹. It seems that W_{cr} remains roughly constant over the lifetime of SNRs, at least in this size and age range, with a value of $W_{\text{cr}} \sim 2 \times 10^{49}$ erg. Assuming that the particle energy density decreases as r_0^{-3} , where r_0 is the radius of the SNR, and assuming also an isotropic particle velocity distribution with $|v| \sim c$, the intensity of cosmic radiation ($E \gtrsim 100$ MeV) at a point within the SNR is $\sim 10^{10}/r_0^3$ erg cm^{-2} sr^{-1} yr^{-1} , where r_0 is in parsec. We choose $E = 100$ MeV as a low-energy cutoff because cosmic-ray particles of energies greater than ~ 100 MeV are not deflected appreciably by the interplanetary magnetic field associated with the solar wind. The calculated intensity would then represent an increase over the current background level¹² of a factor of 10^2 for $r_0 \sim 10$ pc and about 10^3 for $r_0 \sim 5$ pc. Because of the lower initial energy we used, enhancements (an enhancement of 1 implies a doubling of the background level) are less than those calculated by Ruderman¹, but the duration of the exposure of a planet in the situation treated here would be about the lifetime of the SNR, $\sim 10^4$ yr, as opposed to the $\lesssim 10^2$ yr duration of an hypothetical 'pulse' of cosmic rays. In addition, significantly greater enhancements over shorter periods may be expected in the case treated here, because of the non-uniform distribution of particles within the SNR (for example, 'whisks' in the Crab and Cygnus or 'knots' in Cas A¹³).

The model calculations of atmospheric effects correspond to the steady state in this case, because the duration of the event is

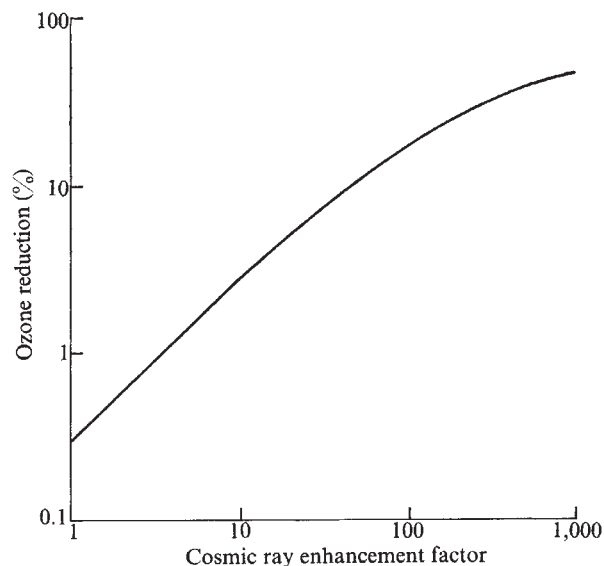


Fig. 1 Reduction (%) of ozone column density in the steady state as a function of the cosmic-ray enhancement factor.

very long compared to the stratospheric recovery time. The calculated global ozone reductions for various cosmic-ray enhancements up to a factor of 10^3 are shown in Fig. 1. It is seen that ozone reductions of ~ 20 – 50% could be expected from enhancements of 10^2 – 10^3 . Although these reductions are smaller than estimated by Ruderman⁷, they are still considerable, especially in the light of their probable long duration.

The waiting time, T_w , for a supernova outburst to occur within a volume $V_0 = (4\pi/3)r_0^3$ of the Galaxy is given by $T_w = T_{\text{Gal}} V_{\text{Gal}}/V_0$, where T_{Gal} is the galactic average time between supernovae in a total volume V_{Gal} . The distribution of supernovae in our Galaxy in space and time is still a matter of some uncertainty. Within 8 kpc of the galactic centre, SNRs seem to lie within a disk ~ 120 pc thick, and from 8 to 14 kpc the thickness is perhaps twice as large and the total volume density is $\sim 1/4$ as great as in the inner regions¹¹. Using these observations and a mean time between supernova events of $T_{\text{Gal}} = 50$ yr, consistent with optical, SNR, and pulsar observations^{14–17}, we have found that at the location of the Earth (10 kpc from the galactic centre), waiting times for extended ($\sim 10^4$ yr duration) ozone depletions of 20–50% are between $\sim 2 \times 10^9$ and $\sim 2 \times 10^{10}$ yr. The probability that such an event occurred near (that is, ~ 5 – 10 pc from) the Earth within the past 10^8 yr is ~ 1 – 5% . Interestingly, the waiting times for such events to occur near a given planet at less than 8 kpc from the galactic centre are much shorter, $\sim 10^8$ and 10^9 yr, respectively.

It thus seems that the ozone depletion effect is significant and long lasting, and may affect the surface biology of the Earth or any Earth-like planet greatly, but is of low probability in our region of the Galaxy. Considering that cosmic and γ radiation does not penetrate to the surface in sufficient doses to affect life in a direct sense¹, and that ultraviolet light from a supernova at several pc is weaker than that from the Sun and of short duration (< 1 yr), the calculated ozone depletion would seem to be the major effect of a supernova on a nearby (~ 5 – 10 pc distant) Earth-like planet.

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Heavy-ion damage in α Fe

We summarise here some preliminary observations of damage in α Fe irradiated with low doses of heavy ions at room temperature. Although the damage produced by such irradiation has been studied in a number of pure metals and alloys using transmission electron microscopy¹, there have been comparatively few studies of materials of technological interest. Iron is of particular interest since it is a major constituent in many practically important alloys including ferritic steels. The use of heavy-ion irradiation (typically to doses $\lesssim 10^{13}$ ions cm^{-2} and energy $\lesssim 200$ keV) provides a convenient method of simulating the displacement cascades generated in metals undergoing fast neutron irradiation. In a majority of the metals and alloys studied the damage consists of a population of vacancy loops produced heterogeneously at the cascade sites by a collapse of the vacancy-rich cascade centres. Interstitial loops are not normally observed at the low doses commonly used, and this is thought to result from the close proximity of the damage region to the foil surface (the mean range of ions is typically ~ 100 Å) which acts as a dominant sink for the mobile interstitial point defects. There have been no previous reports of low-dose heavy-ion damage in α Fe and in other damage experiments² the vacancy component of the damage was not observed.

In this experiment, thin polycrystalline foils of α Fe were irradiated at room temperature with heavy ions of energy between 40 and 240 keV to doses $\approx 5 \times 10^{12}$ ions cm^{-2} . The ions used were Fe^+ , Ni^+ , Ge^+ , Kr^+ , Xe^+ and W^+ , with atomic weights ranging between 56 and 184. The iron used was the same material as that used in ref. 3 where the method of preparation of the specimens and an analysis of the iron purity is given. After irradiation, the foils were examined in a Siemens 102 or a JEM 100B electron microscope. A major experimental difficulty was the appreciable growth of oxide film, even though the specimens were stored under high vacuum (10^{-5} mmHg) after thinning. This, together with problems in producing foils with smooth surfaces and in completely eliminating objective astigmatism, meant that image quality was not as good as can be obtained in other materials. We estimate the visibility threshold for small loops in the best foils to be ~ 20 Å.

A detailed analysis of dynamical black-white images in foils irradiated with 80 keV W^+ ions has revealed the presence of two kinds of perfect loops with $a/2$ $\langle 111 \rangle$ or a $\langle 100 \rangle$ Burgers vectors. All the loops were of vacancy type. The results are consistent with the sequence proposed for the nucleation of interstitial loops⁴: aggregation initially on $[110]$ followed by shear to $a/2$ $\langle 111 \rangle$ or a $\langle 100 \rangle$. The relative numbers of the two kinds of loops appear to depend sensitively on the grain orientation. A full analysis of these results will be reported later.

Information on cascade collapse in α Fe has been obtained from measurements of the efficiency with which various ions produce visible clusters. Results for different 80 keV ions are shown in Fig. 1, where the defect yield (defined as the fraction

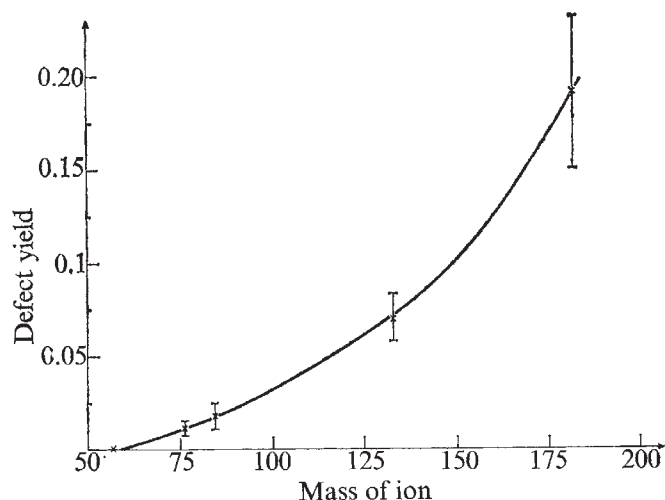


Fig. 1 Plot of defect yield against ion mass for grains close to $\langle 110 \rangle$ bombarded with 5×10^{12} 80-keV ions cm^{-2} .

of cascades which collapse to produce visible clusters) is plotted as a function of the ion mass. No correction has been made for loops slipping out of the foil, but care was taken that grains of similar orientation (in this case near $\langle 110 \rangle$) were selected for defect counting. The relative values of the defect yield should, therefore, be meaningful. Figure 1 illustrates two important results. Firstly, self-ions produce no visible damage; this was true for all ion energies in the range 40–240 keV. Secondly, the defect yield is a strong function of the mass of the incident ions, increasing from zero for Fe^+ ions to $\sim 20\%$ for W^+ ions, the heaviest ions investigated. The loops in α Fe irradiated with 80-keV W^+ ions ranged in size up to ~ 100 Å, but most were much smaller than this, $\sim 90\%$ falling in the size range 20–50 Å.

The absence of observable damage in self-ion irradiated specimens is consistent with results from neutron-irradiated α Fe² which showed that no visible damage is produced at doses $\lesssim 5 \times 10^{18}$ cm^{-2} . This suggests that cascades initiated by self-ions do not collapse, at least at room temperature and above. If this is so, it could have important consequences on the void swelling characteristics of α Fe, as have been discussed in ref. 5.

The explanation for the second observation concerning the sensitivity of the defect yield to ion mass cannot lie in differences in the total number of displacements produced, since, according to calculations⁶, the ions used all produce approximately the same number of displacements, ~ 500 for 80 keV ions (the calculated numbers are shown in Table 1). What does alter, however, is both the spatial distribution of the damage and its depth below the foil surface. We do not consider that the latter affects the cascade collapse. Thus with increasing ion mass cascades become more compact, the vacancy supersaturation is higher and the cascade is hotter (in the sense that the density of energy deposited is increased). The magnitude of the effect is shown in Table 1. The quantities $\langle \Delta X^2_D \rangle$ and $\langle Y^2_D \rangle$ are related to the second moments of the energy distribution function and give measures of the cascade radius parallel and perpendicular to the incident ion direction respectively, while $\langle X_D \rangle$ gives a measure of the mean depth of the damage. These quantities have been computed from the WSS analytical theory⁷; θ_0 is an estimate of the maximum energy density in the cascade, calculated from ref. 8. In going from Fe^+ to W^+

Table 1 Calculations on collision cascades created by 80-keV ions

Ion	Fe	Ge	Kr	Xe	W
Atomic mass	56	76	84	131	184
No. of vacancies in cascade	507	513	518	535	548
$\langle X_D \rangle$ (Å)	193	169	156	105	95
$\langle \Delta X^2_D \rangle^{1/2}$ (Å)	120	102	96	64	59
$\langle Y^2_D \rangle^{1/2}$ (Å)	77	65	60	44	40
θ_0 (eV per atom)	0.21	0.37	0.42	1.26	1.81

ions the volume of the cascade decreases by about an order of magnitude, and the maximum density of deposited energy increases by about the same factor.

The work of English *et al.*⁹ and English (unpublished), has highlighted the difficulty of cascade collapse in another b.c.c. metal, molybdenum. It was found that the defect yield produced by heavy-ion bombardment was dependent on both the irradiation temperature and on the mass of the incident ions. For 60-keV Mo⁺ ions the defect density decreased at irradiation temperatures > 200 °C. This decrease cannot be explained on a vacancy-emission mechanism, where cascades are assumed to collapse athermally and then shrink by vacancy emission, nor by loop loss to the surface. The former mechanism had been successful in explaining the decrease in defect density in copper irradiated at high temperatures with 30-keV Cu⁺ ions¹⁰. It seems that in self-ion irradiation of molybdenum the collapse process is itself temperature dependent. Further experiments with 60-keV Xe⁺ and W⁺ ions have, however, shown that the defect density in these cases remains unchanged at temperatures up to 425 °C, the highest irradiation temperature which could be achieved. It would seem, therefore, that in b.c.c. materials there is a sensitive balance between the collapse of vacancies in a cascade to a vacancy loop or their retaining an uncollapsed distribution and perhaps dispersing in the lattice.

Our results suggest that an important parameter in determining whether or not cascade collapse occurs in α Fe is the energy density or compactness of the cascades. The results from Mo irradiated with heavy ions at high temperatures provide further support for this concept and also suggest that an important and closely associated factor could be the rate at which vacancies diffuse out of the cascade centre into the surrounding crystal. There is considerable doubt regarding the temperature range for long range vacancy migration in α Fe and thus we are not able to separate this factor from the energy density factor in the present results. We plan to clarify this by irradiating iron specimens with ions of different masses at 77 K. The results from pure f.c.c. metals and alloys suggest that energy density in the cascade is a less critical factor governing cascade collapse than for b.c.c. metals. Nevertheless, there is a marked trend for higher cascade collapse efficiencies on going to heavy metals irradiated with self ions, Cu/Cu⁺ $\rightarrow$ Ag/Ag⁺ $\rightarrow$ Au/Au⁺. Moreover, Häussermann¹¹ has shown that in ion-irradiated copper the efficiency of cascade collapse to vacancy loops is higher for Au⁺ ions than for Cu⁺ ions, while Ruault *et al.*¹² have shown that in ion-irradiated gold the nature of the collapsed defects is also a function of ion mass. It seems, therefore, that energy density and cascade compactness are important parameters governing the development of damage structures in irradiated metals and their influence needs to be better understood.

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Mechanical forces of electromagnetic origin

EXPERIMENTS have been reported which verified that at low frequencies a time-varying polarisation P in a dielectric, if taking place in a magnetic field H , results in a mechanical force of density $\dot{P} \times \mu_0 H$. This force is not predicted by the Minkowski energy momentum tensor, but the Abraham form of that tensor gives a force density in an homogeneous isotropic body of magnitude

$$[(\epsilon_r \mu_r - 1)/c^2] \frac{\partial}{\partial t} (E \times H)$$

usually called the 'Abraham Force'. In a dielectric this can be written $\dot{P} \times \mu_0 H + P \times \mu_0 \dot{H}$. It is not surprising that $\dot{P} \times \mu_0 H$ should give a mechanical force, since P corresponds to a movement of electric charge associated with polarisation, but it is difficult to understand why $P \times \mu_0 \dot{H}$ should give a force.

To try to obtain experimental evidence of this effect, the same equipment was used as previously^{1,2}, namely a disk of barium titanate suspended as a torsional pendulum between the poles of a powerful electromagnet, the only change from the previous experiment was to hold P fixed but to vary H with time at the same frequency as the oscillation of the disk. Great difficulties were caused by the magnet system. A considerable coupling of energy to the pendulum resulted from mechanical vibrations in the magnet coils and there may also have been electromagnetic coupling because of small geometrical imperfections in the system. To avoid such resonance effects, we devised the following procedure.

If E and H have the same frequency, then, irrespective of their phase relationship, the time average of the Abraham Force is always zero. Thus if E and H are in time quadrature and if in fact $P \times \mu_0 \dot{H}$ produces no mechanical force, there should be a time-averaged unidirectional force given by $\dot{P} \times \mu_0 H$. In the experiment, E and H were in time quadrature but at a frequency about 250 times the resonant frequency of the pendulum, thereby avoiding parasitic coupling from the magnet system. It was arranged that the direction of the electric field applied across the disk was reversed at the instants when the torsional pendulum reversed in its direction of motion, thereby ensuring that the applied force (if any) would always assist the oscillation of the pendulum. If no oscillation beyond the noise level resulted, the Abraham Force would be verified. In fact, a strong oscillation was observed which agreed closely (within 4%) with the amplitude predicted by the term $\dot{P} \times \mu_0 H$ acting alone.

Full details will be published elsewhere, but we conclude that neither the Minkowski tensor nor the Abraham tensor is consistent with the observed effects.

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High resolution image of copper phthalocyanine

WE report here the successful high resolution of organic material by electron microscopy, a technique extremely sensitive to electron irradiation. As is well known, an inevitable factor that limits such high resolution microscopy is severe radiation damage, and, therefore, the electron beam must be reduced very much below the critical dose to prevent higher order details from fading. As a result, one cannot get high contrast

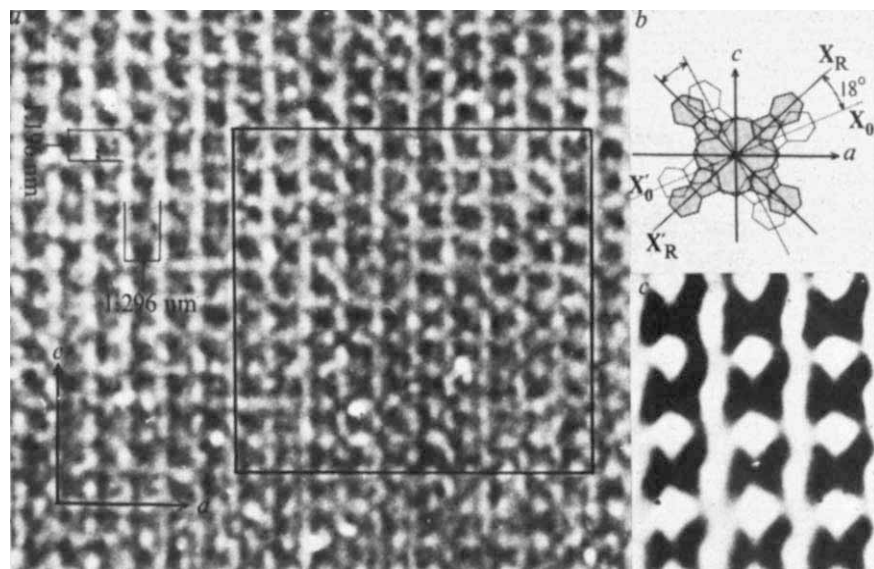


Fig. 1 Thin film for observation was prepared by vacuum deposition on a freshly cleaved KCl substrate, which had been preheated at 400 °C for 1 h. Film thickness was controlled carefully to be < 100 Å. We regard it as a perfect phase object. *a*, High resolution electron micrograph viewed along the *b* axis. Careful inspection reveals some crystal details. *b*, The relationship of the molecular orientation between the averaged image and the assumed isomorphous structure. $X_R X_R'$ indicates the molecular orientation of the retrieved image, $X_0 X_0'$ the assumed molecular orientation. *c*, The averaged image obtained by using the redundancy information from an enclosed region, by an optical method ($\times 1.24$).

by using the usual films, and we used an X-ray film (Ilford, Industrial G) with a high quantum efficiency. With this film, the exposure time did not exceed 5–7 s in conditions of minimal beam irradiation (Williams *et al.*¹).

The specimen used was copper phthalocyanine, a widely used pigment. The thin film specimen for observation was grown epitaxially on a substrate of KCl. Preliminary experiments have found its crystal structure to be of the metastable α form², with crystal parameters: $a = 2.592$ nm, $b = 0.379$ nm, $c = 2.392$ nm, $\beta = 90.4^\circ$, monoclinic, $C2/c$. Furthermore, its crystal structure was found by Ashida³ to be closely isomorphous with platinum phthalocyanine (Robertson *et al.*⁴). The epitaxially grown crystallite has a special orientation relative to the substrate, namely, the *b* axis stands on the substrate surface at an oblique angle of $\sim 32^\circ$, and planar molecules are stacked in parallel along the *b* axis. To observe the molecular image, therefore, the crystallite was tilted by a special holder with an oblique spacer. The observation was carried out in a JEM 100C.

The critical dose of copper phthalocyanine was found to be $1\text{--}2$ C cm⁻² by measuring the complete fading of a diffraction spot (Reimer⁵). High resolution, however, requires many separate diffraction spots to construct the image, and therefore, as stressed above, the electron beam must be reduced below the critical dose. Although the electron dose was not directly measured, it was estimated as ≤ 0.1 C cm⁻² from exposure meter readings.

Figure 1*a* shows a high resolution micrograph, taken at ~ 70 nm under focus. It can be seen that the micrograph is extremely noisy, a result of using minimal beam irradiation and X-ray film with a large grain. We used an objective aperture of 20 μ m in diameter—through which spatial frequencies of up to 1.55 nm⁻¹ could pass. Computer simulation would give the shape of the molecule under optimal focus condition (60–80 nm underfocus) but its shape would be poor⁶. We retrieved the molecular shape by using a lot of periodic unit cells, as described by McLachlan⁷, and Fig. 1*c* represents a retrieved image from the region well defined in Fig. 1*a*, from which an optical diffraction pattern represented the considerable conservation of structure details with a slightly asymmetric intensity distribution from the original electron diffraction pattern. We can see the shape of the molecule clearly in Fig. 1*c*. Figure 1*b* represents the relationship between the averaged image and an assumed structure based on the close isomorphous relationship to platinum phthalocyanine, with a slight rotation of $\sim 18^\circ$.

These pictures strongly suggest that a reconsideration of the crystal structure is in order. We are trying to improve our experiment by combining high resolution micrograph with

intensity data from electron diffraction patterns, as described by Unwin *et al.*⁸.

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Hot lines in the Earth's mantle

THE mantle 'hot spot' or 'plume' hypothesis of Wilson¹ and Morgan² has been used to explain linear chains of volcanic islands and seamounts as well as aseismic ridges. These features of the ocean basins were presumably caused by the motion of lithospheric plates over mantle plumes, since it is assumed that unusually intense volcanism occurs at the Earth's surface above mantle plumes. Initially it was suggested that hot spots do not move relative to the lithosphere or to each other^{1,2}, but it was later proposed that some hot spots undergo a limited amount of motion relative to each other^{3,4}. Here we hypothesise that some linear volcanic chains are the expression not of lithospheric motion over mantle hot spots but of 'hot lines' in the mantle, above which volcanism occurs intermittently.

The idea of mantle 'hot lines' is based on data collected on Easter Island (south-eastern Pacific) and along the tectonic-volcanic lineation running eastwards and westwards from the island. Wilson¹ and Morgan² originally suggested that Easter Island marks the site of one of the major 'hot spots' in the Earth's mantle. The island consists of volcanic rocks, mainly basalt, but with significant quantities of more acidic types, including mugearite, trachyte and rhyolite (ref. 5 and E.B., C.G.A.H., D. E. Fisher, J. Honnorez, J. G. Schilling, J. J. Stipp and M. Zentilli, to be published). The petrology and geochemistry of these rocks (to be published) show close similarities to rocks of other areas interpreted as expressions of mantle plumes, such as Iceland^{6,7}. The Easter Island basalts are richer in alkalis, iron and titanium, and large-ion lithophile elements (such as Ba, Sr

and Zr) than ocean basalts. They are enriched in light rare earth elements, and have $^{87}\text{Sr}/^{86}\text{Sr}$, ^{207}Pb and $^{204}\text{Pb}/^{206}\text{Pb}/^{204}\text{Pb}$ ratios in a range higher than ocean ridge basalts but similar to Iceland basalts (to be published). Volcanism which gave rise to the island occurred less than 2 Myr before present, and mainly within the last 1 Myr (to be published). Thus, the petrology and age of Easter Island are consistent with a mantle plume origin.

If the Easter plume was active in the past, a trail of volcanoes should exist east of the island, along the direction of motion of the Nazca plate, and their age should increase with distance from the island. Indeed, a chain of volcanic islands and seamounts is observed along the so-called Easter Island Fracture Zone (to be published), including the islands of San Ambrosio and San Felix, and extending probably close to the Chile Trench (Fig. 1). The petrology of the islands of Sala y Gomez, San Felix, and San Ambrosio, and of a number of seamounts along the Easter Line, is consistent with a mantle plume origin (to be published). The ages of volcanism along the Easter Line, coupled with the spreading history of the Nazca plate, indicate, however, that the Easter volcanic chain cannot be explained by a fixed, single mantle plume.

On the basis of identification of magnetic anomalies, Herron⁸ demonstrated that the East Pacific Ridge in the south-eastern Pacific became an active spreading centre about 9 Myr BP; before this, the fossil Galapagos Rise (Fig. 1) was an active spreading centre. Anderson and Sclater⁹ reached similar conclusions using the age-depth relationship of Sclater *et al.*¹⁰, even though their model of the spreading history of the Nazca plate differs in some respects from Herron's⁸. Either model would require the development of a gap of several hundred kilometres in the volcanic chain along the Easter line, if a fixed mantle plume under Easter Island is the cause of the volcanic chain (to be published). Our surveys and the topographic map of Mammerickx *et al.*¹¹ indicate that no such gap exists; therefore the Easter volcanic line cannot be caused by a single fixed mantle plume beneath Easter Island. Either a plume existed at the intersection of the Galapagos Rise and the Easter Line when the former was active, and moved to the Easter Island area when the Galapagos Rise became inactive, or two plumes coexisted, one in the Easter Island area, one at the Galapagos Rise (to be published).

The age of volcanism along the Easter line confirms that the volcanic chain could not have originated solely from the motion of the Nazca plate over the Easter Island hot spot. Sala y Gomez was active 1.7 Myr ago, so it could not have formed at the site of Easter Island unless one assumes unreasonably high spreading rates. San Felix and San Ambrosio, located more than 2,700 km from Easter Island, were formed not earlier than one or a few million years ago, and still show signs of activity (to be published).

We conclude that the activity which gave rise to the Easter volcanic line took place intermittently at several sites along the line (Fig. 1). This observation, coupled with the fact that the products of this activity are of the type ascribed elsewhere to mantle plumes, leads us to propose that a thermally anomalous linear zone exists in the mantle beneath the Easter line. This mantle hot line is the cause of the intense volcanism along the Easter line.

We find it unlikely that the Easter line is simply a fracture zone caused by thermal contraction of the lithosphere^{12,13} and that the igneous activity along it is solely the passive result of the lithospheric fracture-fracture zones such as those from the East Pacific (Mendocino, Murray, Clipperton and so on) and from the Atlantic (such as Romanche, St Paul and Vema), presumably caused in part by lithospheric contraction, are characterised by little or no volcanism. The intensity of volcanism along the Easter line, and the fact that the magma source is probably deeper than the source of ocean ridge magmas, suggest that the Easter hot line reflects processes initiated beneath the lithospheric plates.

Based on theory and laboratory experiments, two types of mantle convective flow have been postulated by Richter and

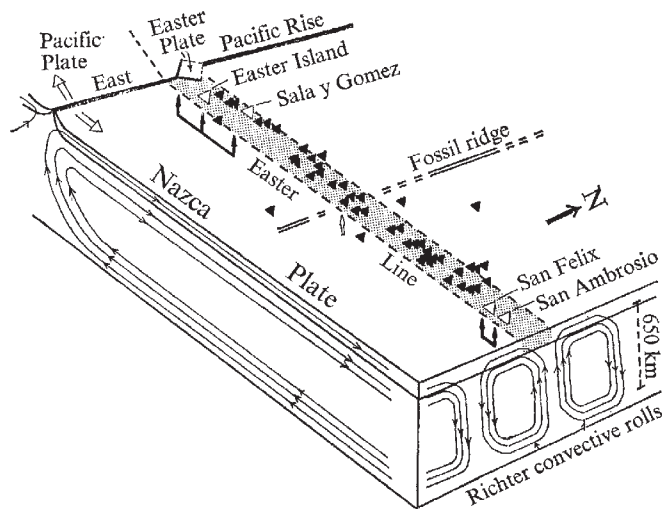


Fig. 1 Schematic illustration of the Easter hot line, showing the distribution of islands and of known seamounts in the area. Vertical full arrows indicate sites of young (< 2 Myr) volcanism; vertical open arrow indicates site of old (~ 10 Myr) volcanism at the intersection of the Easter line and the fossil ridge. The small Easter plate is described by Herron¹⁶. The Easter hot line is shown as due to Richter^{14,15} mantle convective rolls; upwelling spouts^{14,15} may actually be responsible for the line. Δ , Islands; $\blacktriangle$, seamounts.

Richter and Parson^{14,15}. The first is a large scale flow, consisting of the plates themselves and of the return flow necessary to conserve mass, and the second a small scale flow, approaching a Rayleigh–Benard type convection, assumed to reach depths of about 650 km (where a major seismic discontinuity exists), with a similar horizontal scale^{14,15}. The small scale convection rolls tend to become aligned with their axes parallel to the direction of motion of the plate—in times of 20–50 Myr for fast moving plates (10 cm yr⁻¹) but as much as several hundred million years for slow moving plates (2 cm yr⁻¹) (refs 14 and 15).

We suggest that the Easter hot line is the expression of mantle activity occurring along the rising limbs of adjacent Richter^{14,15} convective rolls, with their axes parallel to the direction of motion of the Nazca plate. The required condition of relatively high plate velocity is probably fulfilled or at least approached in the Nazca plate, if we assume that the velocity of plates relative to the sublithospheric mantle is related to the measured spreading rates ($\sim 10 \text{ cm yr}^{-1}$) (refs 8 and 9). Making the same assumption, the direction of the Easter hot line is also the correct direction for a Richter convective roll, as it is almost exactly parallel to the direction of spreading. On the other hand, at lower plate velocities a pattern of mantle flow develops which is characterised by both upwelling and downwelling spouts, also aligned parallel to the direction of plate motion¹².

If the horizontal scale of each cell in the transverse convective rolls is about 650 km (refs 14 and 15), the distance between upwelling spouts would be roughly twice that distance. The San Felix-San Ambrosio 'hot' area is roughly 2,800 km from Easter Island or 2,600 km from a middle point between Easter Island and Sala y Gomez, and the fossil 'hot spot' at the Galapagos Rise was roughly halfway between the Easter-Sala y Gomez and the San Felix-San Ambrosio hot area. Thus, the distance between hot areas along the Easter line (roughly 1,300 km) is consistent with Richter and Parson's model.

We conclude that the Easter hot line is the result of mantle convection rolls or spouts aligned parallel to the direction of plate motion. The small Easter Plate¹⁶ may be the result of complex interaction of two patterns of mantle flow at the intersection of the Easter line and the East Pacific Rise. It is possible that a hot line exists also west of the East Pacific Rise, extending into the Pacific Plate, along a chain of islands and seamounts up to Pitcairn Island. Pitcairn is < 1 Myr old¹⁷.

Hot lines differ from 'normal' fracture zones in that the latter are primarily the result of lithospheric contraction^{12,13}, with the sublithospheric mantle having a passive role and consequently with little or no mantle-derived volcanism. The 'hot lines' are instead caused by activity of the sublithospheric mantle, as in Richter and Parson's model, and are characterised by volcanism of a type previously ascribed to mantle plumes. It is possible that some oceanic aseismic ridges are caused by mantle 'hot lines' rather than by motion of plates over hot spots.

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Acoustic response to chemical stimuli in ground crickets

IN several species of ground crickets (Orthoptera, Gryllidae, Nemobiinae), chemical communication may be used in addition to acoustic communication in bringing males and females together. In laboratory experiments, a high proportion of males of three *Allonemobius* species and one *Pictonemobius* species showed rapid antennation and produced calling songs shortly after exposure to paper from the cages of female crickets. This is the first report of an acoustic response to chemical stimuli in singing Orthoptera.

Populations of *A. fasciatus*, *A. allardi*, *A. tinnulus* and *Eunemobius carolinus* were studied where they occur in sympatry in Suffolk County, Long Island, New York, in 1972, 1973 and 1974. *A. fasciatus* and *A. allardi* were also studied in Tompkins County, New York in 1974. In addition, sympatric populations of *A. fasciatus* and *P. ambitiosus* were studied in Gainesville, Florida, in 1975. Several *A. sparsalsus* males were collected in Florida (by T. J. Walker) and then studied in New York in 1974. The male crickets used in experiments were field captured, except for 33.3% of the *A. tinnulus* males and 18.9% of the *A. fasciatus* males, which were laboratory reared.

Adult males were kept individually in small cages for 1 week before testing. The cage of each male was carried from a rearing area to an experimental area where the cricket was placed in a plastic box (35.5 × 28 × 15 cm) containing one of the following stimuli: (1) control, clean paper towels; (2) paper conditioned in the rearing cages of female crickets (usually 12 virgin females); (3) paper conditioned by males (groups of 8-12 adult male crickets); (4) live females (usually 12 virgin females). All experiments were carried out under laboratory light during the 'on' periods of the rearing room light cycle, at temperatures between 22 and 28 °C. In experiments with paper, a stopwatch

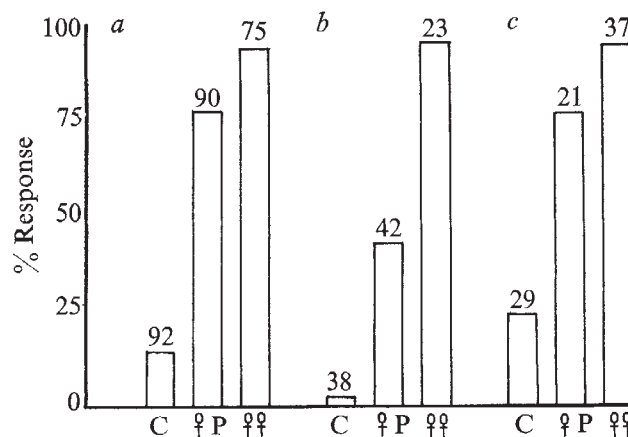


Fig. 1 Proportions of *A. fasciatus* (a), *A. allardi* (b) and *A. tinnulus* males (c) that sang in tests with control paper (C), female-conditioned paper (♀P) and live females (♀♀). For each species, a significantly greater proportion sang on female-conditioned paper than on control paper. A significantly greater proportion of *A. allardi* males sang in the presence of live females than on female-conditioned paper. Numbers above bars refer to number of males tested.

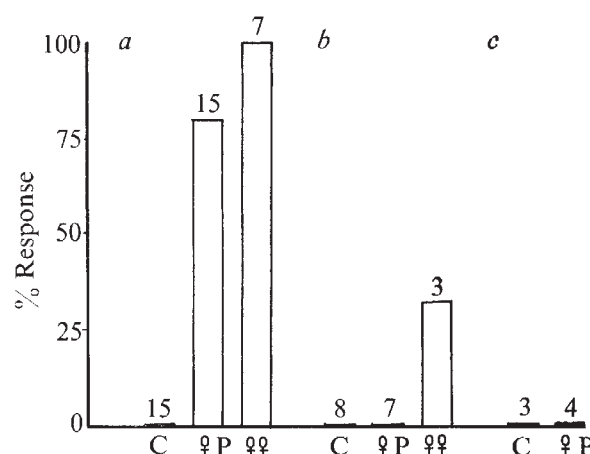
was started when the male was dropped into the box; in experiments with live females, timing was begun when the male made antennal contact with a female. The watch was stopped at 5 min, or when the male began to stridulate. A stridulatory response within 5 min was noted as a positive response. Each male was tested only once with a given stimulus; for stimuli other than controls, males were tested only once every 24 h.

In addition to the main series of experiments, *A. fasciatus* males were tested with paper from the cages of early instar and older male *A. fasciatus* juveniles (that is, juveniles without visible ovipositors).

Many of the males gave a vigorous positive response in tests with paper conditioned by females (Figs 1 and 2). These males walked in the test box for several seconds after being placed on the paper, slowly antennating and palpating the substrate. Often, the cricket showed an obvious increase in the rate of antennation and made frequent erratic changes in body orientation, as though searching. Soon, usually within seconds, the male raised his tegmina (forewings) and stridulated. Some males stood still and sang, others slowly walked while singing.

A wide range of behaviour was shown by males that did not respond with song to conditioned paper. Some were completely inactive in the test box, others made the transition from ex-

Fig. 2 Proportions of *P. ambitiosus* (a), *E. carolinus* (b) and *A. sparsalsus* males (c) that sang in tests with control paper (C), female-conditioned paper (♀P) and live females (♀♀). A significantly greater proportion of *P. ambitiosus* males sang on female-conditioned paper than on control paper. Numbers above bars refer to number of males tested.



ploratory walking to the quick searching behaviour, but produced no song within 5 min.

The numbers above the bars in Figs 1, 2 and 3 are the numbers of males tested with each stimulus; the height of the bars shows the proportion of males that sang within 5 min. For *A. fasciatus*, *A. allardi*, *A. tinnulus* and *P. ambiguus*, the proportion of males that sang in response to paper conditioned by conspecific females was significantly greater than the proportion that sang on the control paper ($P < 0.05$ in 2×2 G tests of independence¹; Figs 1 and 2). In the absence of visual, acoustic, or tactile cues, males of the four species sang in response to some factor, presumably chemical stimuli left on the substrate by females. None of the *Eunemobius carolinus* and *A. sparsatus* males gave a positive response in the presence of conspecific female paper.

A. fasciatus, *A. allardi*, and *A. tinnulus* males showed a significantly greater response to conspecific female paper than to paper conditioned by conspecific males (Fig. 3). Furthermore, *A. fasciatus* males showed a greater response to paper conditioned by adult females than to paper conditioned by *A. fasciatus* juveniles (Fig. 3). These results suggest a sexual role for chemical communication in these species. Ground crickets of these genera produce quiet, high pitched songs relative to those of field crickets (subfamily Gryllinae), or even other nemobiine genera (for example, *Eunemobius*)². The softer, higher pitched songs are not likely to carry far to attract females at a distance. Perhaps the song-releasing stimuli left on the substrate by females are pheromones which indicate areas where singing males are most likely to attract females. The stimuli may be important in species isolation; I have experimental evidence of specificity at the species level².

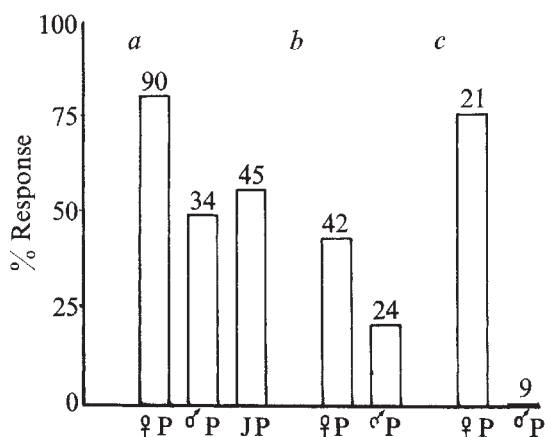


Fig. 3 Proportions of *A. fasciatus* (a), *A. allardi* (b) and *A. tinnulus* males (c) that sang in tests with female-conditioned paper (♀P), male-conditioned paper (♂P), and juvenile-conditioned paper (JP) for *A. fasciatus*. For each species a significantly greater proportion sang on female-conditioned paper than on male-conditioned paper. A greater proportion of *A. fasciatus* males sang on female-conditioned paper than on juvenile-conditioned paper. Numbers above bars refer to number of males tested.

Previous studies have described pheromone-like communication in crickets^{3,4} and chemically-induced stridulation has been reported for the Douglas fir beetle⁵. The data reported here, however, demonstrate song-releasing chemical stimuli in insects well known for their acoustic communication. Further studies may reveal something about the evolutionary origins of cricket song. In some cockroach species (for example, *Blattella germanica*) males raise their wings and expose gustatorial glands (which females feed on) in response to antennal contact with a female pheromone^{6,7}. This is of interest here since crickets are believed to have a cockroach-like ancestor⁸.

At present, the origin and nature of the song-releasing stimuli are unknown. Initial attempts to isolate stimuli from the faecal pellets of females have resulted in ambiguous bioassays.

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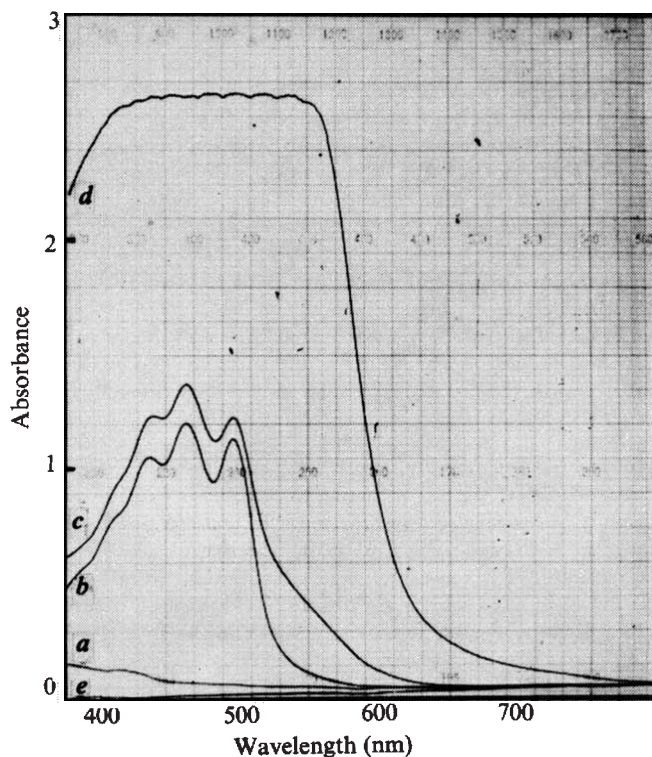
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Changeable coloration of cornea in the fish *Hexagrammos octogrammus*

ALTHOUGH fish are considered 'lower' vertebrates, the visual apparatus of some species has a high degree of adaptability unparalleled in other vertebrates. For example, members of the Hexagrammidae can change their cornea rapidly from colourless in the dark, to deep red in bright light^{1,2}. We have studied this ability in *Hexagrammos octogrammus* Pallas, a common shallow-water fish of the Japan Sea, and found that it is caused by the effects of illumination on the distribution of coloured cytoplasm in the corneal chromatophores.

Specimens of *H. octogrammus* caught by night or kept in the dark have a cornea as transparent and colourless as that of most fish. But specimens collected in their natural environment by day, or kept in an illuminated aquarium, have a bright orange or deep red cornea. If a light-adapted fish is placed in the dark, its cornea gradually pales, becoming

Fig. 1 Absorption spectra measured in pupil zone of corneas from *H. octogrammus* in different adaptation states: a, in full darkness; c, intermediate (yellow) coloration observed after about 30 min in sunlight after full dark adaptation; d, full coloration after 2 h in sunlight; b, yellow cornea of *Pleurogrammus monopterygius* in full colour; e, control.



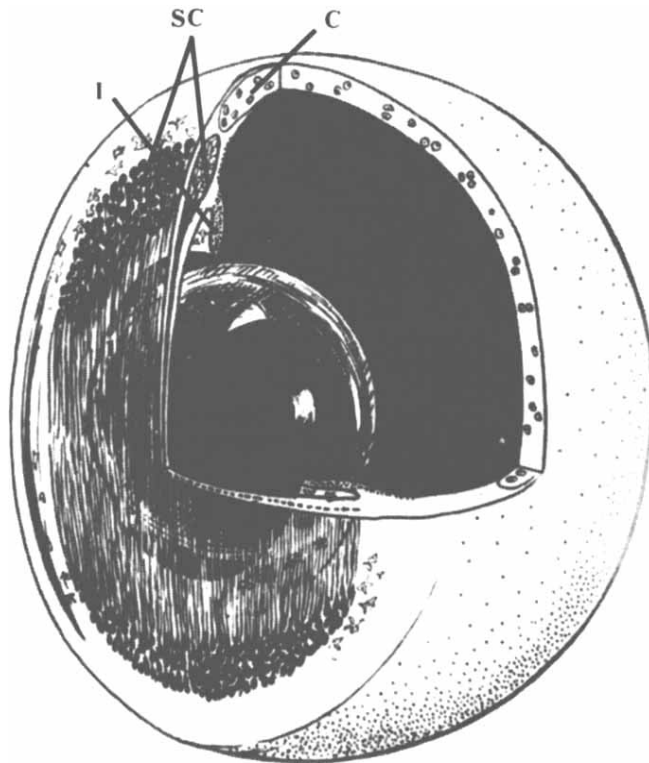


Fig. 2 Reconstruction of anterior part of the eye in *H. octogrammus*. SC, Chromatophores; C, chondreal sclera; I, iris.

ing yellow and then colourless. Similarly, in bright illumination, a colourless cornea becomes yellow and then red, even at night. The speed of colour change depends on the intensity of illumination, and when a fish is transferred from direct sunlight to darkness, complete decoloration usually takes 1.5–2 h. If the fish is kept continuously in light intensities of about 10 lx, its cornea becomes yellow without reddening.

The cornea of *H. octogrammus* has surprising absorptive properties as a colour filter. Its transmittance in the pupil zone in the light-adapted state does not exceed 0.3% for wavelengths of 400–500 nm. It absorbs more than 10% of the light up to a wavelength of 580 nm (Fig. 1). The most saturated coloration reported so far for a fish cornea has been that of the South American *Astronotus* (Cichlidae)³—yet in this species the minimum transmittance is not less than 4%, absorption above 10% being observed only for wavelengths shorter than 510 nm.

This changing corneal coloration can be compared with retinomotor and pupil reactions, which regulate the quantity of light reaching the receptor layer. The most likely function of the yellow and orange coloration is to improve the resolution of the eye by eliminating harmful loss of contrast of retinal image due to (1) a blue veil of light scattered on intraocular and extraocular media, and (2) chromatic aberration⁴, which can be quite significant in the fish eye⁵. Thus shallow-water fishes like *H. octogrammus* searching for food on a well-lit substratum can benefit from a coloured cornea in spite of the loss of sensitivity because it is only at low light intensities that preretinal filters become a disadvantage. The exceptional saturation of the filters in *H. octogrammus* is tolerated because the fish can, so to speak, remove its sunglasses when it gets dark.

In most animals there is diffuse coloration of some part of the eye—in tree shrew, ground squirrels and diurnal snake the whole lens is yellow⁶, in others, as in some squid⁶.

by specialised corneal chromatophores, which differ considerably in structure from ordinary dermal chromatophores.

Chromatophore cell bodies comprise two compact sickle-shaped masses near the upper and lower edges of the cornea, outside the pupil zone. In a vertical section of the cornea, cell masses are surrounded by fibrous tissue. Each chromatophore (50–70 μm in diameter) is pear shaped and gradually tapers into a single, flattened process. All processes are oriented vertically (in the eye of a living fish) and extend downwards or upwards, according to the position of the cells in the upper or lower cell mass, and overlap the pupil zone (Fig. 2).

Corneal coloration is altered by redistribution of coloured cytoplasm between cell bodies (Fig. 3) and their processes. Direct observation of the living fish under a dissecting microscope showed that in fish kept in bright light the processes are filled with cytoplasm, but in the dark they become empty and invisible.

There are two kinds of corneal chromatophore—deep red and yellow—resembling dermal erythrophores and xanthophores in their coloration. When a dark-adapted fish is exposed to bright light, the processes of the yellow chromatophores fill up first, giving a transient yellow colour to the cornea (Fig. 1c). The corneas of *Pleurogrammus monopterygius* and *H. stelleri* (both Hexagrammidae) have only yellow chromatophores, the colour of their corneas in the light-adapted state being similar to the intermediate coloration of that of *H. octogrammus* and the permanent colour of the cornea of pike and perch⁷. The absorption bands at 427, 455 and 485 nm reveal the carotenoid nature of the yellow pigmentation. The coloration of red cornea of *H. octogrammus* in light-adapted fish corresponds to absorption in the red corneal chromatophores, their absorption spectrum consisting of a single broad band (the half-band about 400–550 nm) with a single flattened maximum at

Fig. 3 Ultrastructure of the chromatophore from *H. octogrammus* cornea. Numerous lipid granules (LG), arborised invaginations of cell membrane (I), collagen fibres (C), pinocytosis vesicles (PV), mitochondria (M) and Golgi apparatus (GA) are visible. ($\times 29,750$).



about 480 nm. The difference between the absorption spectra of the two types of corneal chromatophore, shown by microspectrophotometry, cannot be explained as resulting from differences in concentration of the same coloured substance.

The corneal chromatophores differ from other (dermal) chromatophores in having only one process, and having, in the cytoplasm of the cell body and process, homogeneous round granules of intermediate electron density (100–120 nm in diameter), which might be lipid droplets in which carotenoids are dissolved (Fig. 3). Both cell bodies and processes contain many microtubules (25 nm in diameter), lining the axis. No lipid granules have been found in the processes of dark-adapted fish, although microtubules and numerous pinocytosis vesicles are sometimes present. There are many invaginations of the cell membrane, up to 100 nm wide and several μm long, with pinocytosis vesicles attached to them. Unlike ordinary dermal chromatophores—erythrophores and xanthophores—the corneal chromatophores contain no pterinosomes⁸.

Microtubules are directly concerned with dispersion and aggregation of pigment granules in melanophores^{9,10}, and antimetabolic agents (colchicine and vinblastine) which destroy the normal function of microtubules may block the movement of pigment granules¹¹. After injection of colchicine into the ocular bulb of *H. octogrammus* the response of cornea to a change of illumination is slower and weaker.

Changes of the corneal coloration caused by specific corneal chromatophores are not restricted to members of the Hexagrammidae. They have been observed, but to a lesser degree in several species of other families, such as the Blennidae, Cottidae and Tetraodontidae.

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The experiments described here show that this is the case: the function of the aversive response seems to be protection of the starfish against nematocyst discharge on contact with coral.

The role of nematocyst toxins was investigated by comparison of the effects on *A. planci* of extracts of whole coral tissue and homogenised isolated nematocysts from three coral species, including *Millepora dichotoma* ('fire coral'), which is only rarely attacked by *A. planci*. To validate comparisons between the extracts of each species of coral, and show up any differences in activity, it was necessary to equalise concentrations of non-stimulatory components of the extracts. Collins⁵ found that only low molecular-weight components of coral extract, apparently amino acids, elicited arm withdrawal. It was assumed that soluble protein represented the bulk of non-stimulatory material in both nematocysts and tissue cells, and so the protein concentrations of the extracts were equalised—by dilution of the more concentrated extracts—after estimation by the method of Lowry *et al.*⁶.

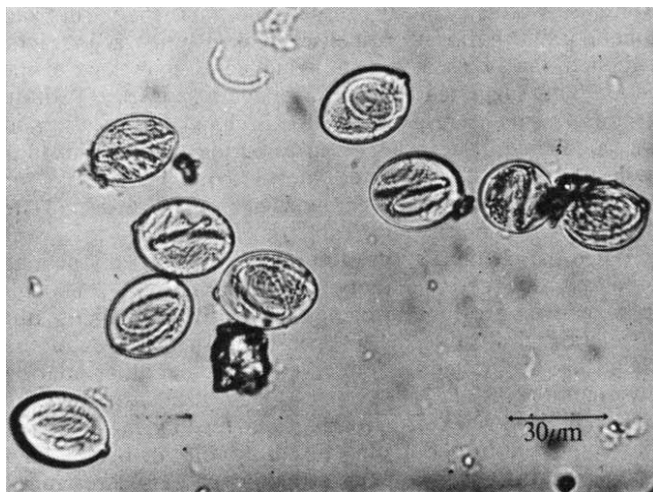
Table 1 summarises the effects of dripping extracts near the tips of arms of moving starfish. It shows that the homogenised nematocyst preparation of only one coral, *Millepora dichotoma*, evoked stronger responses than those produced by the corresponding whole coral extract, and even then the responses evoked were weaker than those produced by the whole tissue extract of *Acropora multicaulis*, in spite of the far greater protein concentration of the *Millepora* extract.

Moreover, the high efficiency of isolation of nematocysts of *Millepora dichotoma* (Fig. 1) made possible an estimate of the proportion of total tissue volume in intact *Millepora* occupied by nematocysts: it was approximately 2%. Thus the contribution made by nematocyst contents to the arm-rearing and tube-foot retraction activity of a whole coral extract seems to be negligible.

We even found that intact isolated nematocysts had no observable effect on the tube feet of *A. planci*—yet accidental splashes of nematocyst suspensions on to our skin produced typical stinging sensations.

These unexpected results suggested that nematocyst contents had leaked into the whole coral extracts during preparation, although most nematocysts remain undischarged. This objection was negated by testing extracts of mesenterial filaments, which contain greater concentrations of nematocysts than other coral tissues (Fig. 2) and may be removed intact from corals with large polyps. Even so, as Table 1 shows, the responses elicited by the mesenterial

Fig. 1 Photomicrograph of nematocysts isolated from 'fire coral', *Millepora dichotoma*, and used in the preparation of nematocyst extracts. The darker objects are fragments of coral skeleton. For further explanation, see text.



Aversive behaviour of crown-of-thorns starfish to coral evoked by food-related chemicals

WHEN *Acanthaster planci*, the coral-predating crown-of-thorns starfish, encounters living coral or when it is presented with coral extracts, it rears the arms near to the stimulus source aborally and retracts the tube feet of these arms into the ambulacral groove^{1,2}. This aversive behaviour has generally been attributed to the effect of discharge of coral nematocysts¹⁻³, or to toxins released from nematocysts⁴. But arm rearing may be evoked before contact is made with corals, and both "withdrawal" responses are produced by non-coral food or food extracts which stimulate *A. planci* to feed (R.J.M., unpublished). This suggests that it is not nematocysts or their toxins which are responsible, but rather chemicals from coral tissue.

Table 1 Comparison of the effects on *A. planci* of extracts of whole coral tissue, homogenised nematocysts and mesenterial filaments

Coral	% Purity (approximate) of nematocyst preparation	Extract	Protein concentration ($\mu\text{g ml}^{-1}$)	Mean response, % (to 5 drops and 10 drops, respectively)			
				Arm rearing		Tube-foot retraction	
<i>Acropora multicaulis</i>	60	Whole coral extract	100	8,	42	8,	33
		Homogenised nematocyst supernatant		0,	0	0,	8
<i>Porites solida</i>	50	Whole coral extract	73	0,	17	8,	17
		Homogenised nematocyst supernatant		0,	0	8,	8
<i>Millepora dichotoma</i>	85	Whole coral extract	680	0,	25	8,	25
		Homogenised nematocyst supernatant		8,	33	8,	33
<i>Fungia fungites</i>	—	Whole coral extract	62	25,	58	33,	67
		Mesenterial filament leachings		8,	42	8,	42
		Homogenised filament supernatant		17,	42	17,	42
<i>Platygyra lamellina</i>	—	Whole coral extract	85	33,	67	25,	33
		Mesenterial filament leachings		17,	42	8,	42
		Homogenised filament supernatant		8,	25	0,	42

Extracts of whole coral tissue were prepared by immersion of living corals in distilled water at 5 °C for 12 h, followed by centrifugation to remove cell debris, zooxanthellae and nematocysts, rotary evaporation to dryness, and redissolution in seawater. To isolate nematocysts, uncentrifuged whole coral extracts were fractionated by differential sedimentation to separate nematocysts from cell debris and zooxanthellae. The nematocyst preparations were homogenised in seawater to discharge them and break up the capsule, and then centrifuged. Corals were induced to extrude their mesenterial filaments by scrubbing, and the filaments were collected using forceps. They were then left overnight in filtered seawater at 5 °C, shaken to break up the structure, and centrifuged: the supernatant was tested as "leachings". The third extract was produced by homogenising the centrifugate—consisting of remaining tissue and nematocysts—followed by centrifugation. The supernatant was tested as "homogenised filament supernatant". The extracts were tested on four starfish by dripping at a rate of about one drop per s on to spots 1 cm from three arm-tips of moving starfish, avoiding dripping directly on to tube feet. The mean responses are to 5 drops and 10 drops, delivered concurrently, and were calculated by grading the strengths of responses on a four-point scale (0 to + + +), averaging, and rounding off to the nearest whole number. As far as possible, the same animals were used for testing different extracts of the same coral species.

filament extracts are, in nearly all cases, weaker than those produced by whole coral extracts.

It thus seemed clear that neither nematocysts nor their contents were primarily responsible for the arm and tube-foot "withdrawal" responses evoked by coral extracts, although the nematocysts in living coral may have some effect. This suggested that the aversive responses constitute at least partly a response to food-related chemicals, and we conducted a search for arm-rearing activity in low-molecular-weight compounds known to be responsible for feeding attraction in other marine invertebrates⁷⁻⁹. The results are summarised in Table 2.

When the chemicals were tested individually, only betaine produced arm-rearing activity at concentrations low enough to suggest its presence in coral as an active ingredient. But a synergistic effect seems to operate when mixtures of the chemicals are tested, as observed in some other marine invertebrates⁷⁻⁹.

The link between arm rearing and feeding in *A. planci* was confirmed by the finding that a mixture of betaine and three amino acids potent at inducing arm rearing—arginine, proline and glutamic acid—induced stomach

Fig. 2 Photomicrograph of a fragment of a mesenterial filament from the brain coral *Platygyra lamellina*, showing the high concentrations of nematocysts present. Two types may be distinguished where the nematocysts have been ejected from the surface of the filament by the pressure of the slide coverslip.



eversion in five out of nine starfish tested, using Collin's assay for feeding-inducing activity.

To examine for the presence of betaine and the amino acids in coral tissue, the ninhydrin-positive fraction of the low molecular-weight fraction of an extract⁴ of *Acropora* sp. was run in several sample strips alongside betaine standards and standard mixtures of the amino acids, on a paper chromatogram in a descending medium of butanol-acetic acid-water (3:1:1). When developed with a stain for alkaloids¹⁰ it revealed a spot on the coral fraction strip running in the same position as betaine. The eluate of part of another sample strip corresponding to the alkaloid spot induced arm rearing in only two out of six trials, but the eluate of the rest of the strip possessed no activity. It thus seems likely that betaine, or another alkaloid with similar R_f in the solvent used, is the most potent factor in eliciting the arm-rearing response.

On another sample strip on the chromatogram, staining with ninhydrin revealed spots which corresponded with the position of the amino acid standards: thus their presence in coral tissue is also very likely.

Thus we found that arm rearing in *A. planci* can be induced by chemicals present in coral tissues which also stimulate it to feed. A consideration of the morphology of asteroids leads us to expect some raising of arm-tips in response to chemicals derived from prey, to aid the tube feet in location of epifaunal prey, and this is borne out by the literature¹¹⁻¹⁴. In *A. planci*, however, the full response seems to be exaggerated beyond that of other asteroids, the arms being raised and curled back along their entire length.

Although our results suggest that nematocysts are directly responsible only to a limited extent for the aversive reaction of *A. planci* when it encounters living coral, it is certainly under threat from them, as discharged nematocysts have been found attached to tube feet after contact with coral tentacles (R.J.M., unpublished). It seems likely that the exaggerated arm curling, triggered by chemicals derived from coral tissue, serves to protect the tube feet against excessive nematocyst discharge on contact with coral. While this protection cannot be complete, the

strength of the nematocyst defences of a coral species may not be as important as has been suggested (refs 1 and 2 and unpublished results of R. F. G. Ormond, H. J. Hanscomb and D. H. Beach) in determining the acceptability of that species to *A. planci* as prey.

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Hybrid crown-of-thorns starfish (*Acanthaster planci* x *A. brevispinus*) reared to maturity in the laboratory

THE coral predators *Acanthaster planci* L. and *A. ellisii* (Gray) have been the subject of many publications in recent years. But there is another little known *Acanthaster* species, *A. brevispinus* Fisher, which was described from two specimens dredged in the Sulu Archipelago, Philippine Islands¹. The species has been reported from the Great Barrier Reef region, Queensland^{2,3} and specimens have been collected recently off Townsville, North Queensland, from sandy substrates and not on coral reef. Although *A. brevispinus* differs conspicuously from the other species in having very short and numerous spines on the aboral disk surface, Madsen⁴ and Caso⁵ were dubious of its status in their reviews of the genus *Acanthaster* Gervais. Phenotypic variation of *A. planci* was suggested in verbal discussion among Australian biologists. We describe here a resolution of the status of *A. brevispinus* by *in vitro* crosses with *A. planci* which led to the rearing of hybrid starfish.

Ripe specimens of *A. brevispinus* were collected by trawling in December 1973, near the time of annual gamete release by *A. planci* in Great Barrier Reef waters⁶. Gonads were dissected from starfish of both taxa. Ovaries were treated with 10^{-5} M 1-methyladenine in seawater to mature the oocytes⁷, which were fertilised *in vitro* at 28 °C with sperm suspensions. Four groups of larvae, that is, each species and reciprocal crosses, were reared from eggs using the technique which gave a high success rate for *A. planci* larvae⁸. The different larvae passed through similar bipinnaria and brachiolaria stages. Numbers of late stage larvae in each batch represented 10-20% of the original numbers of eggs, except for the *A. brevispinus* batch in which only a few normal late brachiolariae developed. After 4 weeks, late larvae were transferred to static conditions with filamentous substrates of coralline algae, coral skeleton and filamentous algae.

The newly metamorphosed starfish were five-armed and

Table 2 Arm rearing in response to food-related chemicals and mixtures

Substance	% Arm rearing at			
	10^{-2} M	10^{-3} M	10^{-4} M	10^{-5} M
Lysine	10	0		
Arginine	40	0		
Tryptophan	70	0		
Histidine	20	0		
Hydroxyproline	0	0		
Proline	50	0		
Glutamic acid	80	0		
Betaine	—	100	65	0
Lactic acid	90	0		
Trimethylamine	100	0		
Taurine	90	0		
Trimethylamine oxide	100	0		
Mixture				
10^{-3} M Proline				
10^{-3} M Arginine				
10^{-3} M Glutamic acid				
10^{-3} M Tryptophan				
10^{-4} M Betaine				
10^{-4} M Proline				
10^{-4} M Arginine				
10^{-4} M Glutamic acid				
10^{-4} M Tryptophan				
10^{-6} M Betaine				

Solutions were tested by dripping 10 drops at about one per s about 1 cm from an arm tip of a moving starfish. Ten or 20 trials were performed for each solution and individual starfish were generally tested twice. The results are expressed as the percentage of trials in which rearing was observed.

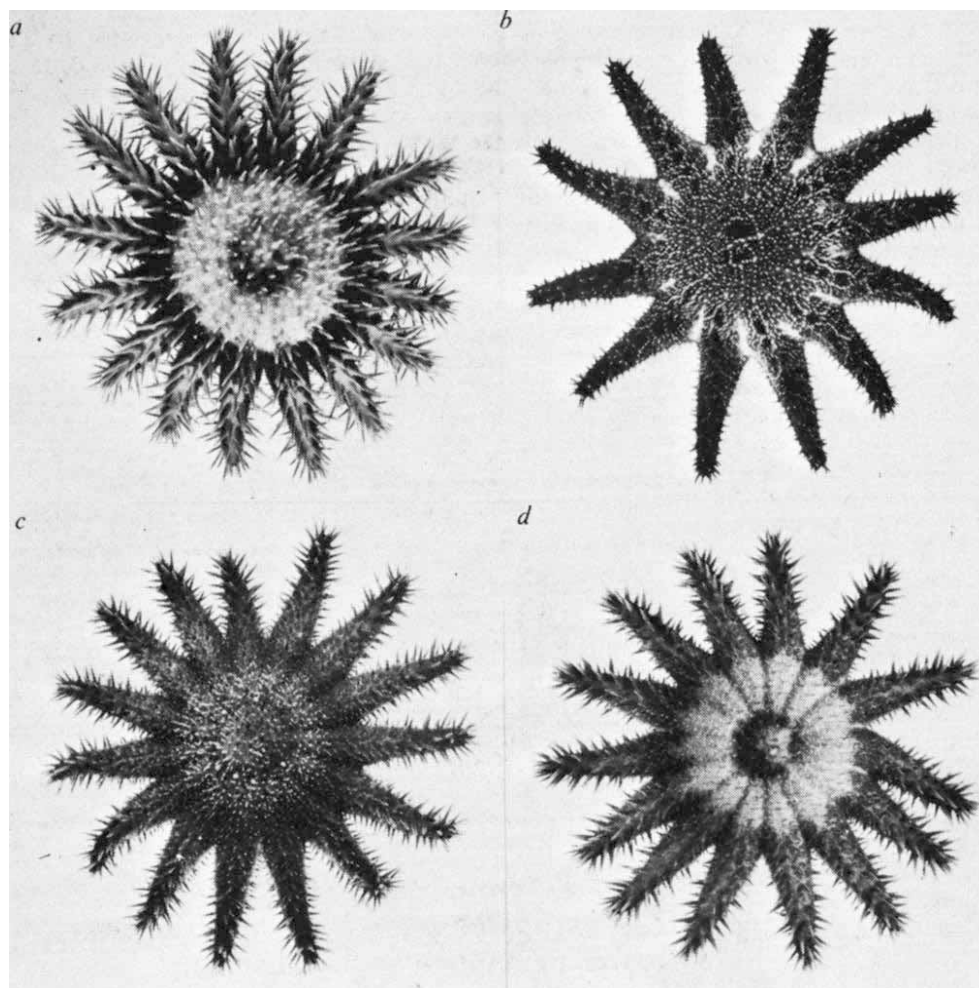


Fig. 1 Aboral views of starfish. a, *A. planci* (215 mm diameter); b, *A. brevispinus* (225 mm); c, *A. planci* hybrid (210 mm); d, *A. brevispinus* hybrid (210 mm).

0.4–1.0 mm in diameter. They fed on encrusting algae. Many failed to develop normally and, 6 weeks after metamorphosis, 60 *A. planci*, 30 *A. planci* hybrids, six *A. brevispinus* hybrids and no *A. brevispinus* remained. (For brevity, hybrids are named after the maternal taxon.) At this time, the young starfish were transferred to aquaria in a recirculating seawater system 33–35‰ salinity and $25 \pm 3^\circ \text{C}$, and provided with coralline algae on coral skeletons for food. The three kinds of young starfish grew and added arms in the order described for *A. planci* by Yamaguchi⁹. Five to seven months after metamorphosis they achieved their adult number of arms and about this time commenced feeding on coral polyps, *Pocillopora damicornis* and *Acropora acuminata*, provided in addition to coralline algae. Late in their second year of development, the hybrid starfish and *A. planci* developed gonads with normal gametogenesis (sampled by biopsy). Sizes at 1 and 2 yr of age are shown in Table 1.

It is impossible to distinguish hybrid starfish and *A. planci* by morphology during the early months of development. But hybrids with a diameter of more than 200 mm have conspicuously shorter spines than *A. planci* and longer spines than *A. brevispinus* (Figs 1 and 2). In other features in which *A. planci* and *A. brevispinus* differ consistently,

the hybrid starfish show intermediate conditions, that is, density of aboral disk spines, length of aboral pedicellariae, length and number of adambulacral spines, shape of subambulacral spines, interradial marks on the disk and taper of arms. The intermediate hybrid features may be more similar to one parental condition, that is, on the aboral disk surface of hybrids, spine length approximates more to the *A. brevispinus* condition (Fig. 2), while the pedicellariae are slender, resembling those of *A. planci*. There are no consistent differences between *A. planci* hybrids and *A. brevispinus* hybrids. Some *A. planci* show a persistent "bull's-eye" pattern on the aboral surface from a pale ring of papulae (dermal evaginations) (Fig. 1a). One of three *A. brevispinus* hybrids shows the pattern (Fig. 1d); none of twelve *A. planci* hybrids shows the pattern.

A. planci and *A. brevispinus* differ in habitat and morphology and also in their feeding behaviour. Scallops, *Amusium balloti* and *Amusium pleuronectes*, occur in the habitat of *A. brevispinus*, and in the laboratory *A. brevispinus* traps and eats live scallops beneath the disk. It shows an arched posture when digesting live and dead scallops and scallop flesh. *A. planci* never traps live scallops and never arches when feeding on scallop flesh. (*A. planci*

Table 1 Diameters of starfish, *A. planci* and hybrids, reared in the laboratory

	1-yr-old		2-yr-old	
	Mean diam. (mm)	Range (mm)	Mean diam. (mm)	Range (mm)
<i>A. planci</i>	33.4	18.0–61.2 (17)	198	145–255 (13)
<i>A. planci</i> hybrid	30.8	19.7–44.1 (8)	199	165–250 (9)
<i>A. brevispinus</i> hybrid	36.4	35.7–37.0 (2)	223	215–230 (3)

Numbers of individuals measured are given in parentheses.

Table 2 Reactions of starfish, *Acanthaster* species and hybrids, to live scallops

	Nos of Individuals	Recorded hours of activity	Mean contacts with scallop per h of activity
<i>A. planci</i>	3	43.6	0.50
<i>A. planci</i> hybrid	5	107.0	1.94*
<i>A. brevispinus</i> hybrid	3	62.9	2.31*
<i>A. brevispinus</i>	3	19.9	3.52

The three *A. brevispinus*, 220–240 mm diameter, were tested 3d after collection. The others were laboratory-reared starfish, 160–190 mm diameter, which had been fed solely with algae and coral before this experiment. Each starfish was placed in a glass aquarium 30 × 30 × 50 cm with running seawater and continuous illumination. Activity was recorded by an 8-mm movie camera, situated above the aquarium, making exposures at 30-s intervals. The starfish was accustomed to the apparatus for a day and then a live scallop, *Amusium pleuronectes*, was added and filming commenced. Duration of filming was 27–31 h. Film strips were analysed frame by frame. Each new contact with the scallop by the starfish, that is, passing at least part of an arm over the scallop, and periods of quiescence were recorded. Data for each starfish were recorded as numbers of contacts per unit period (h) of locomotion in case contacts with scallops were purely incidental, being related only to the amount of activity. Numbers of 0, 1 . . . 6+ contacts per hour were accumulated for each kind of starfish. For statistical analyses, these numbers were adjusted to 100 h total activity and the numbers in each category for two kinds of starfish compared by χ^2 analysis.

*These data are not significantly different ($P < 0.5$); they are the only data for two kinds of starfish which are not significantly different ($P < 0.001$) (see explanation of statistical analysis above).

normally feeds on hermatypic coral, but it will accept a range of animal flesh when hungry.) The hybrid starfish trap scallops and they may adopt an arched posture, although not as pronounced as *A. brevispinus*, when feeding on scallops. Further evidence of behavioural reactions to scallops by hybrid starfish, inherited from their *A. brevispinus* parent, is shown in Table 2. Hybrid starfish reared on coral showed a significantly greater rate of contacts with scallops than *A. planci* of similar experience. Contacts with the scallop usually occurred when the starfish moved towards it across the floor of the aquarium and contact was often terminated by the scallop escaping with rapid valve movements.

It has been shown that the starfish described as *A. brevispinus* are genetically different from typical *A. planci*: crossing them produces starfish which differ in morphology and behaviour from *A. planci* reared in the same conditions. There is no evidence of gamete incompatibility between the two taxa, as found in interspecific crosses attempted with other asteroids^{10,11}. There is obviously a high degree of genetic compatibility between them, at least to F₁ hybrids. But they are isolated in the field by their habitat preferences, *A. planci* on coral reefs and *A. brevispinus* in deeper water on sandy substrates. Although probably they both spawn during summer in Great Barrier Reef waters, the chance of cross fertilisation is remote: ripe *A. planci* spawn in response to a pheromone released with gametes by neighbouring individuals¹² and this mechanism is unlikely to operate over hundreds or thousands of metres, with consequent dilution of the pheromone and gametes. No natural intermediate forms have been found in the Great Barrier Reef region,

although the reef environment has been surveyed extensively by biologists aware of the morphology of *A. brevispinus*^{13,14}.

A. planci and *A. brevispinus* are taxa which do not interbreed in a region of sympatry. They are species retaining a high degree of genetic compatibility, suggesting recent speciation. It is notable that the morphological differences between *A. planci* and *A. brevispinus* are much greater than the morphological variation of *A. planci* throughout its wide Indo-Pacific distribution and between *A. planci* and the eastern Pacific taxon, *A. ellisii*, which is of disputed status^{15,16}. If degree of morphological difference within this genus even approximately reflects degree of genetic difference, then all populations of *A. planci* and *A. ellisii* are genetically compatible. These suggestions are in accordance with a recent evolution of *A. planci* and dispersal through the Indo-Pacific region. It seems more likely that *A. planci* evolved from an ancestor like *A. brevispinus* than vice versa. *A. planci* is atypical of coral reef asteroids in its large size and often conspicuous mode of life. It is more probable that an atypical, specialised, coral predator evolved from an unspecialised, sand inhabitant than vice versa. In this case, development of large sharp spines from the small blunt spines of *A. brevispinus* gave the means of protection from the numerous predators which are a particular hazard of the habitat and mode of life adopted by *A. planci*.

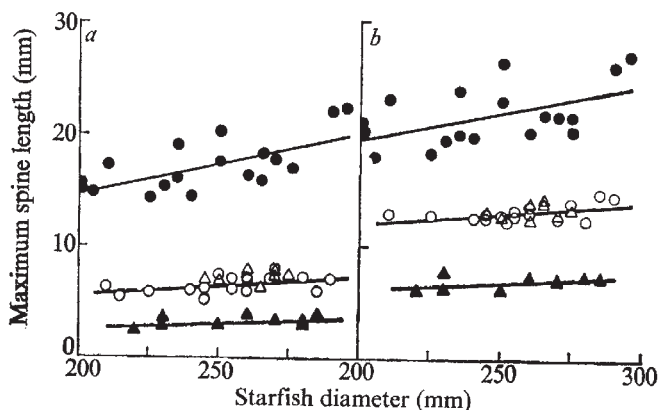
This is the first time that asteroid hybrids have been reared successfully in the laboratory. The only other reported cross fertilisations resulted in abnormal embryos or malformed early larvae¹⁰. In contrast, there is an extensive literature on echinoid hybridisation with crosses involving quite divergent taxa¹⁷. Although almost no echinoid hybrids have been reared beyond plutei, hybrids from reciprocal crosses between the sand dollars *Dendraster excentricus* and *Encope californicus* have been reared to young urchins and in these hybrids paternal characters predominate¹⁷. In *A. planci* and *A. brevispinus* hybrids, characters of neither parent predominate and the hybrids generally show intermediate conditions.

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Fig. 2 Maximum disk (a) and arm (b) spine lengths on the aboral surfaces of *Acanthaster planci* (●), *A. brevispinus* (▲), *A. planci* hybrids (○) and *A. brevispinus* hybrids (△). Regression lines were fitted by least squares method (hybrid data pooled).



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Cultivation of larvae of Japanese eel

ALTHOUGH the breeding place of the European eel has been detected¹, the morphology and ecology of their larvae, especially in pre-leptocephalus stages, still remain to be investigated. We have, however, succeeded^{2–4} in obtaining some pre-larvae of the Japanese eel, *Anguilla japonica*, following artificial induction of maturation and spawning in aquaria. The larvae survived for only 6 d; their teeth characteristic of muraenoid larvae were merely in the state of anlagen and their eyes were completely devoid of retinal pigment. Further information of the early development of the eel is therefore required to enable elucidation of their life history. Here, we describe the larvae of Japanese eel developing for up to 14 d in the laboratory.

Silver eels were collected in the Hiranuma and Mabuchi Rivers in Aomori prefecture (Japan) in September 1975. Females and males were matured with repeated injections of chum salmon pituitary homogenate and Synahorin (Teikoku Zoki), respectively^{5,6}. Eggs were stripped from the females, placed into glass dishes and inseminated by the dry method with the sperm of 2 or 3 males. The eggs were kept in seawater at 23 °C until they hatched. The larvae were reared at 23 °C on the day of hatching. Subsequently the larvae were maintained at 19 °C.

The larvae survived for 14 d. The following description is based on the observation of the larvae from days 7 to 14 after hatching. Since larvae of that period of development are very weak and their tail is liable to shrink a little during microscopic observations, the measured values of body length and the myomere number of larvae

may be approximate. On day 7, the mean body length was ~6.2 mm, and on day 14, ~7.0 mm. The number of myomeres was 53–54 + 50 (pre- + postanal myomeres) 7–10 days after hatching and 54 + 55–60 in those 11–14 days after hatching.

The larvae on day 7 had well developed tooth anlagen on the jaws and a large ventriculus cerebri in the brain. A broad embryonic fin enveloped the compressed body continuously from the posterior region of the head to the caudal margin of the vent. There was no trace of pigmentation except in the caudalmost part of the membranous fin. By day 8, some black retinal pigment began to appear in both eyes (Fig. 1a) and after a further 12 h pigmentation of the eyes was fully developed. During that time the ventriculus cerebri became smaller. These larvae, unlike the earlier stages⁴, were seen to swim in a horizontal position and to rest suspended in the water in a head-down attitude. From days 9 to 11 after hatching, both the upper and lower jaws developed considerably, and the tooth anlagen came to differentiate into definite teeth, which lengthened gradually, one after another. By day 12, sharp teeth were observed to protrude obliquely from the jaws (Fig. 1b). An oil drop near the cranial end of the yolk sac became extremely small. On day 14 after hatching, black pigmentation was still seen in the larvae, but only in the eyes and on the caudalmost tip of the membranous fin (Fig. 1c). A pair of pectoral fins were present. The mouth which was directed downwards during the foregoing stages took a forward direction. The upper jaw had three pairs of shorter teeth in addition to the longest, grasping tooth, while the lower jaw had four pairs of teeth (Fig. 1d). The lower jaw moved occasionally, but the mouth did not close perfectly.

The most advanced larvae obtained seem to resemble closely the smallest European eel larvae collected by Schmidt⁷ in the Atlantic Ocean. From these findings on both the European and the Japanese eel, a clearer outline of the morphological changes of the eel during the initial stage of its life cycle has been obtained.

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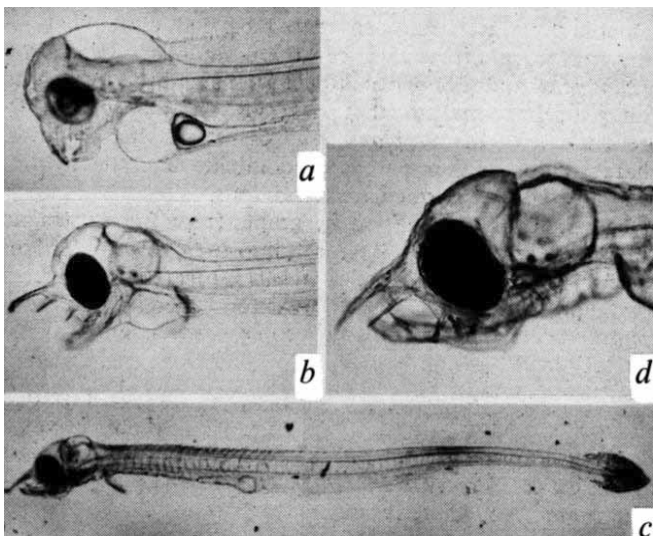
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Genetics of expression of xenotropic virus and autoimmunity in NZB mice

CERTAIN retroviruses (RNA tumour viruses) have been implicated in the autoimmune disease of New Zealand mice¹. These mice produce large numbers of xenotropic retroviruses^{2,3} and contain high concentrations of the retroviral envelope antigen gp 69/71 in their serum and tissues⁴. Moreover, gp 69/71 and the corresponding antibodies contribute to the immune deposits in the nephritic kidneys of NZB and (NZB × NZW)F₁ mice⁴. Nevertheless, it is not established that xenotropic viruses are the primary cause of the autoimmune disease of NZB mice. Conceivably, these agents may be involved only secondarily. The presence of RNA viruses in NZB mice may explain neither the production of antibodies against DNA, nucleoproteins, and erythrocyte antigens nor the anomalies of T- and B-lymphocyte function⁵. Transmission of autoimmunity has not been achieved

Fig. 1a. Head of larva, day 9 after hatching, × 25; b, head of larva, day 12 after hatching, × 22; c, larva, day 14 after hatching, × 13.5; d, head of larva, day 14 after hatching, × 38.5.



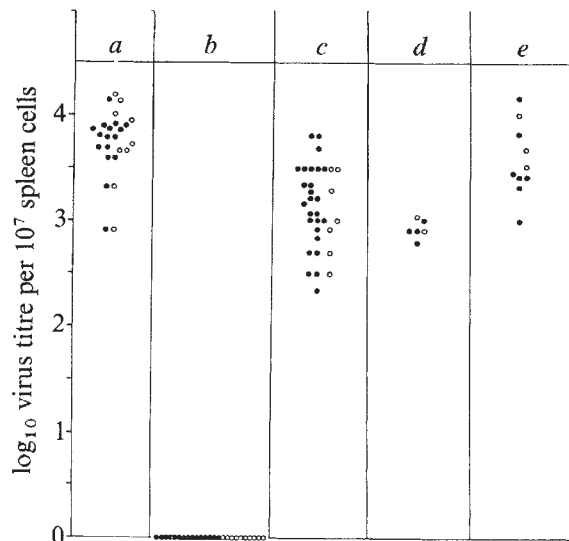


Fig. 1 Xenotropic virus titres in NZB and SWR mice and their crosses. All parental strains of mice were obtained from Jackson Laboratory, Bar Harbor, Maine. Spleen cell suspensions were prepared from the test animals as described previously¹⁰ and assayed on the 81 cell line¹¹ for xenotropic virus as follows. The 81 cells were grown and the assay was carried out in McCoy's 5A medium (Grand Island), supplemented with antibiotics and 15% foetal calf serum. The 81 cells (2×10^6) were seeded into 6-cm plastic Petri dishes. The next day the medium was withdrawn and the cells were treated with 2 ml DEAE dextran in McCoy's 5A medium without serum ($25 \mu\text{g ml}^{-1}$) for 30 min at 37°C . Serial dilutions of spleen cell suspensions of 5 ml McCoy's 5A medium were inoculated on the 81 cells after the DEAE dextran was rinsed away. On days 3 and 10 after inoculation 3 ml medium was added (similar results were obtained when on day 0 the spleen cells were inoculated in 10 ml medium and the cultures left undisturbed until day 7). On day 7 the medium was changed completely. Foci were counted on unstained dishes on days 13–14 after infection. Titres are expressed in this and succeeding Figs as $\log_{10}$ of the number of foci induced per 10^7 inoculated spleen cells. In experiments not shown here we found that the number of foci that developed increased linearly and proportionally to the number of virus-producing spleen cells inoculated. Satellite foci did not occur in the dishes containing lower dilutions of the virus-producing spleen cells. Each circle represents an individual animal. ●, Male; ○, female. *a*, NZB (1–9 months); *b*, SWR (2–11 months); *c*, (SWR $\times$ NZB) F_1 (1–9 months); *d*, (NZB $\times$ SWR) F_1 (2 weeks); *e*, (SWR $\times$ NZB) F_1 $\times$ NZB (3–5 months).

with cell-free filtrates from NZB mice⁶, nor by transplanting NZB lymphomas⁷ which are known to produce large numbers of virus particles⁸. Only spleen cells from old NZB mice can transmit the autoimmune disorder⁶. Transmission of the disease with cell-free extracts is unlikely to succeed because the NZB xenotropic virus cannot productively infect mouse cells; it can infect only cells of heterologous species^{2,3}. Here we deal

with the vertical transmission of NZB viral genes as Mendelian traits—a phenomenon that has been demonstrated with other retroviruses⁹—to test the hypothesis that the development of autoimmunity in NZB mice and their crosses is independent of the expression of high titres of xenotropic viruses. We have found that the high grade expression of infectious xenotropic virus characteristic of NZB mice is a genetically determined trait. Two independently segregating loci seem to specify the NZB phenotype. So far, animals that are virologically like NZB have failed to develop signs of autoimmune disease.

Infectious xenotropic virus was measured by a modification¹⁰ of the test described by Fischinger *et al.*¹¹. Spleen cells from the test animal were cocultivated on monolayers of the 81 clone of the murine sarcoma virus-transformed CCC feline cell line (provided by Dr Peter Fischinger)¹¹. Xenotropic viruses of various origins replicate in the 81 cell line and produce foci which are proportional in number to the input of virus-producing cells. This quantitative assay detected xenotropic viruses produced by cells of different mice, including BALB/c, B10.A, C57BL/6, AKR and C57L (Fig. 2 and unpublished observations). The assay reflected the virological status of the spleen cells on the day they were inoculated on the 81 cell line because mitomycin treatment of NZB spleen cells just before inoculation did not reduce the titre of xenotropic virus relative to that of untreated NZB spleen cells. Thus, the assay reflected the *in vivo* virological status (phenotype) of the animal, as was demonstrated in the case of ecotropic virus-producing strains¹⁰.

Figure 1 shows the expression of xenotropic virus in two strains, NZB and SWR. As found by others^{2,3}, xenotropic virus was present in high titres in all the NZB mice (age range 1–9 months), whereas in none of the 25 SWR mice (2–11 months) could xenotropic virus be detected. It seems that the production of infectious xenotropic virus by SWR mice is either 'switched off' or proceeds at an extremely low rate. Alternatively, SWR mice may produce a defective virus. In any case, the 81 cell line assay clearly distinguished between NZB and SWR phenotypes: the former were uniformly positive in high titre and the latter uniformly negative.

Figure 1 also shows that all (SWR $\times$ NZB) F_1 , (NZB $\times$ SWR) F_1 and (SWR $\times$ NZB) F_1 $\times$ NZB backcross mice expressed high titres of xenotropic virus early in life. This indicates inheritance of the NZB virus phenotype by a genetic mechanism that is dominant, highly penetrant and contributed equally well by both sexes. No linkage to the sex of the animal was detected. We also tested the expression of infectious xenotropic virus in other crosses: (C57BL/6 $\times$ NZB) F_1 , (B10.A $\times$ NZB) F_1 and (AKR $\times$ NZB) F_1 (Figure 2). In all cases a picture similar to the (SWR $\times$ NZB) F_1 cross emerged—dominant, highly penetrant expression of the NZB viral phenotype.

Tests of the first backcross with SWR and the F_2 generations showed clearcut segregation that was independent of the maternal direction of the cross (Fig. 3). In the SWR backcross progeny, the ratio of virus-positive to virus-negative mice was

Table 1 Autoimmune markers and disease in NZB mice and their crosses with C57BL/6(B6), SWR and B10.A mice

Strain	Age (months)	Direct Coombs' test	Anti-DNA antibody		Nephritis
			Native	Denatured	
(B6 $\times$ NZB) F_1 male	6–7		0/6	0/6	0/14
	9	0/9	0/14	0/14	0/14
(SWR $\times$ NZB) F_1 male	6–7		0/14	4/14*	0/18
	8	0/8	0/8	1/8*	0/8
(B10.A $\times$ NZB) F_1 male	8	0/4	0/4	0/4	0/13
female	7		0/7	1/7*	0/7
NZB male	8–12	6/8	3/8	8/8**	3/8
	3–5	0/7	0/7	7/7**	0/7

Direct Coombs' tests were carried out according to the method of Long *et al.*¹⁵. Antibodies to native and denatured DNA in the sera of the mice was quantified by the ^{14}C DNA/glass filter technique developed by Lewis *et al.*¹⁶. Percentage binding of denatured DNA in the positive serum samples of the F_1 hybrids* was in the range 10–18% (mean 12%), whereas among the NZB mice** it was 14–36% (mean 25%).

Nephritis: degree of glomerular involvement was determined by histological criteria of Toniatti *et al.*¹⁷, using haematoxylin-eosin and PAS-stained sections of kidneys.

approximately 3:1 (51:18, $P > 0.75$). This suggests two independently segregating loci either of which gave a positive phenotype. According to this interpretation, the virus-negative mice were the result of simultaneous homozygosity of recessive alleles to these two autosomal genes. Results obtained in the F_2 generation tended to support this interpretation, as a 15:1 ratio (54:4, $P > 0.9$) was obtained. So far, no linkage between coat colour and virus expression has been found. Linkage with other traits is being sought.

The quantitative data further support a multiple independent gene model because intermediate titres of virus (up to 150 focus-forming units (FFU)) were found in some mice of the backcross and F_2 generations. In contrast, all the F_1 mice expressed high titres of virus. The segregation ratios for high:intermediate:negative virus phenotype in the backcross and F_2 mice suggest that one of the NZB loci is responsible for high grade virus expression when present alone or in association with the other virus-inducing locus. Let us call the first locus NZV_1 and the second NZV_2 for convenience. The ratios suggest that when NZV_2 is present by itself, intermediate expression of virus would result. This hypothesis is being tested by crossing intermediate-titred mice with each other or with SWR mice to determine if the effect is caused by a segregating locus. If this is so, the hypothesis of phenotypic mixing between the NZB virus and a presumed defective SWR virus to give intermediate titres can be rejected. It is also conceivable that the high and intermediate titres are the result of two different types of NZB xenotropic viruses. This is being analysed.

The virological patterns in the various F_1 hybrids were quantitatively and qualitatively similar, indicating that no major genetic influences inhibited the expression of NZB virus. There was a minor effect in that $(B10.A \times NZB)F_1$ hybrids tended in general to have lower titres than the other two hybrids.

Although the data fit a two-locus model and are incompatible with a single-locus model, we cannot exclude that more than two virus-inducing loci are segregating. If there were one locus with high penetrance and several other loci with low penetrance, the overall segregation ratios might mimic a two-locus pattern. Further studies of backcross families will clarify this point.

We cannot state whether the postulated NZB loci are genetic elements of the virus itself or are genes that promote the expression of the virus. The distinction between these alternatives requires the use of genetic markers that distinguish

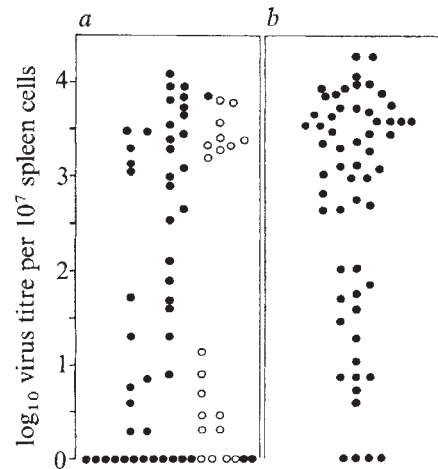


Fig. 3 Xenotropic virus titres in mice of first backcross with SWR and the F_2 generations. All mice were 3–4 months old. a: ●, $SWR \times (SWR \times NZB)F_1$; ○, $(SWR \times NZB)F_1 \times SWR$. b, $(SWR \times NZB)F_2$.

between subtypes of xenotropic viruses (for example, NZB-xenotropic virus, or MuLV- x^B , and C57BL/6-xenotropic virus, or MuLV- x^A)¹². It is likely that we are not dealing with either the presence or absence of genetic information required for the phenotype under study since the low virus strains C57BL/6, B10.A and AKR obviously can make xenotropic virus; and the SWR mouse, although 'virus negative' when tested in the 81 (cat) and A673 (human)¹³ cell lines, is known to have the p12 of xenotropic virions in its cells¹³.

Table 1 shows the results of preliminary studies for autoimmune disease in NZB mice and their crosses with SWR, C57BL/6 and B10.A mice. Markers of autoimmunity were readily detected in NZB mice. In contrast, these abnormalities were either absent or infrequent in all of the F_1 crosses tested, although all the animals expressed high titres of xenotropic viruses from an early age. These data do not take into account the possibility that autoimmune markers or autoimmune disease may develop later in the F_1 hybrids than in the NZB parent. This time-dependent phenomenon has been noted by others and is a complicating feature of genetic analysis of autoimmunisation in NZB mice and their crosses¹⁴. Since the material required for virological analysis was obtained in all of the mice by biopsy of the spleen, these animals are currently being aged and will be retested for signs of autoimmunisation at a later time. It will also be of interest to note if the virus-negative backcross and F_2 progeny develop autoimmune disease later in life. Such a finding would support the hypothesis that the autoimmune abnormalities of NZB mice are independent of xenotropic viruses.

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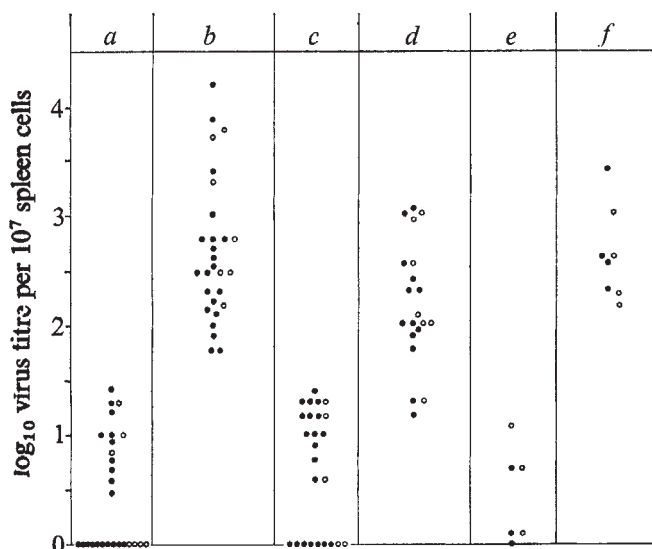
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Fig. 2 Xenotropic virus titres in C57BL/6, B10.A and AKR mice and their F_1 hybrids bred by crossing with NZB. ●, Male; ○, female. a, C57BL/6 (8–9 months); b, $(C57BL/6 \times NZB)F_1$ (6–9 months); c, B10.A (5–9 months); d, $(B10.A \times NZB)F_1$ (5–8 months); e, AKR (4–6 months); f, $(AKR \times NZB)F_1$ (2–7 months).



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Sex differences in formation of anti-T-cell antibodies

A NATURALLY occurring thymocytotoxic autoantibody (NTA) has been found in significant titre early in life in NZB mice¹. This antibody is present in almost all NZB mice by 3 months of age, and is associated with an age-dependent loss of T cells in these mice^{2–4}. NTA may be responsible for an initial loss of suppressor T cells in NZB mice and thereby contribute to the development of autoimmunity. Although other mouse strains (NZW, C57BL/6J, AKR/J, BALB/cJ, and 129) may produce NTA later in life, the prevalence and titre of NTA is much lower than in NZB mice. NTA reacts with brain- and thymus-derived cells as well as thymocytes, but not B cells or non-lymphoid abdominal organs^{1–5}. In contrast to standard anti-θ serum, NTA can combine with both Thy 1.1 (θ-AKR) and Thy 1.2 (θ-C3H)^{1,6}. Genetic factors have been implicated in the development of autoimmunity in New Zealand mice^{7,8}. In addition, X-linked immune response genes have been described for synthetic⁹ and naturally occurring¹⁰ nucleic acids. Moreover, female hybrids (NZB/NZW) develop a more severe disease than males. We therefore undertook the present study to determine the contribution of the X chromosome to the development of NTA.

Hybrid mice were produced by crossing NZB/N and DBA/2 strains. The DBA/2 strain was chosen because of the reported lack of warm-reacting NTA at approximately 1 yr of age². Since the presence of NTA in high titres occurs earlier in the life of NZB than in other strains, 12-month-old hybrid mice were chosen for study so that differences in the formation of NTA would not represent time differences alone, but genetic background as well. Because the X chromosome of the NZB strain may be important in determining the presence of NTA, we wished to rule out a simple hormonal mechanism. To study this (DBA/2 × NZB/N) and (NZB/N × DBA/2)F₁ hybrid virgin mice of both sexes were castrated at 5 weeks of age¹¹, with littermates left intact. Sera obtained at 1 yr of age were studied for the presence of NTA as described previously, with only minor modifications⁶. Briefly, thymocytes from 4–6-week-old C57BL/6N were incubated with 30 μCi ⁵¹Cr per 10⁷ cells for 30 min at 37 °C. Labelled thymocytes (5 × 10⁴) were added to 50 μl of serially diluted test serum and incubated for 30 min at room temperature followed by 30 min at 4 °C. Cells were

spun, washed twice and incubated with 50 μl 1:15 rabbit complement (previously absorbed with C57BL/6N thymocytes) for 30 min at 37 °C. The suspension was then centrifuged and 50 μl of supernatant removed and counted. The positive control was a pool of NZB sera with known NTA activity; the negative control consisted of sera from 1-yr-old female DBA/2 mice. Maximum ⁵¹Cr release was obtained by freeze-thawing, and cytotoxicity of complement (C) alone determined. Percentage cytotoxicity was calculated as

$$\frac{(\text{c.p.m. test serum} - \text{c.p.m. C})}{(\text{c.p.m. freeze-thaw} - \text{c.p.m. C})} \times 100$$

All sera were assayed in duplicate with good reproducibility. The cytotoxic titre was the last dilution giving greater than 50% ⁵¹Cr release. A positive titre was at least 1:4.

Approximately half of the unmanipulated female NZB × DBA mice had NTA at 1 yr of age, whereas none of the males was positive (Table 1). Castration of the females did not affect the percentage positive or the titre of NTA. Castration of the males at 5 weeks of age, however, led to NTA formation indistinguishable from that of females (Table 1).

DBA-mothered F₁ hybrids had a lower incidence of NTA than corresponding NZB-mothered mice. Nevertheless, the same pattern of NTA incidence was observed. Hybrid males failed to produce NTA unless they were castrated.

The studies of uncastrated mice demonstrate NTA formation in female but not male F₁ hybrids. Superficially, the results for the DBA-mothered F₁ hybrids might suggest a role for an immune response gene carried on the NZB X chromosome, but not the DBA X chromosome. This would be in agreement with a previous study of the anti-DNA response¹⁰. According to this model, NZB-mothered F₁ males would receive a 'responder' X chromosome and produce NTA. We found, however, that NZB-mothered F₁ males failed to produce NTA, indicating a mechanism other than an X-linked immune response gene. Moreover, the observation that castration abolished the difference between hybrid males and females, suggested a sex hormonal mechanism. Thus, the lack of NTA in male NZB hybrids is due in part to a functional testis; the possibility that male sex hormones are responsible is under investigation. It is not clear why NZB males develop NTA. They are relatively infertile and may have a deficiency in suppressive male sex hormones. Alternatively, additional factors (such as a more rapid loss of suppressor cells than occurs in NZB/DBA male F₁ hybrids) may operate to make it possible for them to develop NTA. In addition, both NZB male and female mice share similar severity of disease manifestations unlike the NZB/NZW F₁ model where females clinically have a more severe expression of autoimmunity^{8,11}.

The data reported here provide a possible explanation for the female predominance in both murine and human autoimmunity. Male sex hormones may inhibit the production of certain autoantibodies; therefore, males would be protected from the development of autoimmunity. The study of such a mechanism may lead to a better understanding of autoimmunity as well as potential therapeutic intervention.

Table 1 NTA at 1 yr of age

F ₁ hybrid	Sex	Positive NTA test	
		Number/total	%
♀ × ♂			
NZB × DBA	♀	11/20	55
NZB × DBA	♀, castrated	9/16	56
NZB × DBA	♂	0/15	0
NZB × DBA	♂, castrated	10/18	56
DBA × NZB	♀	2/8	25
DBA × NZB	♀, castrated	2/7	29
DBA × NZB	♂	0/6	0
DBA × NZB	♂, castrated	2/7	29
Parental strain			
NZB	♀	10/10	100
NZB	♂	10/10	100
DBA	♀	0/8	0
DBA	♂	0/8	0

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Serum containing endotoxin-induced tumour necrosis factor substitutes for helper T cells

WHEREAS immunocompetent precursor B cells recognise antigenic determinants by way of specific surface receptors (an interaction which presumably initiates cell division^{1,2}), the binding of antigen to surface receptors alone does not result in the differentiation of B cells to antibody-secreting cells. To accomplish this differentiation step a second signal is needed. This signal may be provided by T cells in the form of a soluble factor which they produce *in vitro* after contact with antigen³⁻⁵. Besides T cells, activated peritoneal macrophages have also been shown to produce a factor which allows B cells from athymic Nu/Nu mice to produce antibody against fowl gammaglobulin *in vitro*. Production of this factor is increased by addition of bacterial lipopolysaccharide (LPS) to the culture medium⁶. LPS itself has been shown to provide the second signal (antigen being the first) for antibody production by B cells⁷, but it is not clear whether the effect on antigen-reactive B cells is direct, or mediated by other cells. We report here that injection of LPS, in certain conditions in the mouse, induces the release of factors into the serum which enable B cells to produce antibody to heterologous red-cell antigen *in vitro* in the absence of helper T cells. The factor is released in large quantity when LPS is injected into mice that have been primed with BCG. It is hardly, if at all, detectable in the serum of mice injected with LPS but not pretreated with BCG. Our investigation was prompted by the discovery that serum from BCG-infected mice given LPS causes acute necrosis of established tumour grafts in syngeneic mice (tumour necrosis serum, TNS)⁸. After partial purification, tumour-necrotising activity was shown to reside in a glycoprotein fraction (termed tumour necrosis factor, TNF) with a molecular weight of about 150,000 that migrates with the α -2-globulins⁹.

TNS used in the experiments reported here was produced in CD-1 Swiss mice. Twenty million living BCG organisms (Trudeau Institute, Saranac Lake, New York) were injected intravenously, followed by intravenous injection of 25 μ g LPS (Difco) at the peak of hepatosplenomegaly, usually 2 weeks later. The mice were exsanguinated 2 h after injection and their serum separated and passed through a Millipore filter before use in tissue culture.

The production of haemolytic antibody by spleen cells from BDF₁ mice was studied in the Mishell-Dutton culture system¹⁰. Initial experiments showed that the production of antibody to sheep red blood cells (SRBCs) by unfractionated spleen cells was accelerated and augmented by addition of TNS or TNF to the culture medium (Table 1). This led to the question of whether TNF acted directly on B cells, or indirectly through macrophages or T cells. Experiments with fractionated spleen cells were therefore carried out. After removal of macrophages by passage through Sephadex G-10 columns¹¹ the spleen cells failed to produce antibody to SRBCs. Responsiveness was restored by addition of 5×10^{-5} M 2-mercaptoethanol (2-ME)¹², but not by addition of TNS. In Sephadex-treated cultures supplemented with 2-ME, addition of TNS enhanced the generation of antibody-forming cells (Fig. 1). As the *in vitro* response to SRBCs requires the participation of helper T cells, experiments were carried out to determine the effect of TNS on T-cell-depleted populations. Spleen cells which had been depleted of T cells by treatment with anti-Thy-1.1 serum and complement (after passage through Sephadex G-10 columns) produced only few plaque-forming cells (PFCs) in culture; the production of antibody to SRBCs by the macrophage

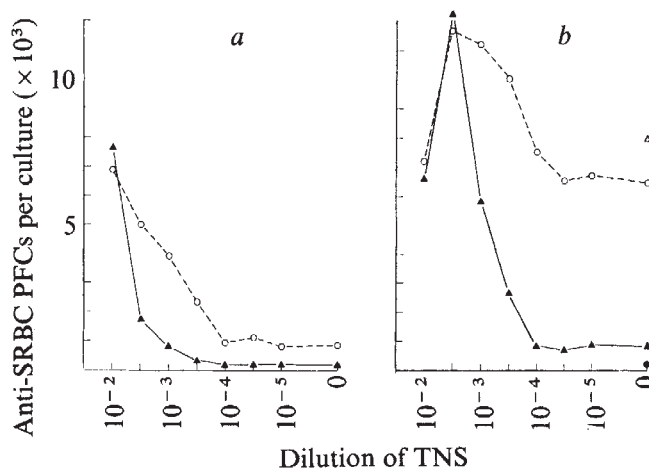


Fig. 1 Restoration of antibody production by macrophage- and T-cell-depleted spleen cell cultures with TNS. PFC response on days 3 (a) and 4 (b). Each point is the average of 2 cultures. ●, 2-ME not added; ○, 2-ME added; ▲, 2-ME added, T cells removed by treatment with anti-Thy-1.1 and rabbit complement; △, 2-ME added, cells treated with rabbit complement (control).

and T-cell-depleted spleen cell population was restored by addition of TNS to the culture medium (Fig. 1).

Figure 2 shows results of experiments with congenitally athymic Nu/Nu mice. Since they lack helper T cells, spleen cells from these mice do not produce antibody to red blood cells *in vitro*. Addition of TNS to the culture medium permitted a response (of the same magnitude as that of spleen cells from littermate Nu/+ mice which do not lack T cells) to the antigen that was used to sensitise the cultures (burro red blood cells, BRBCs), but not to an unrelated antigen (SRBC). Addition of TNS was effective even if delayed as long as 2 d (Table 2), and this was also true for TNF. Thus, injection of LPS into BCG-infected mice causes release of a factor which can substitute for helper T cells in the production of antibody to heterologous red-cell antigen *in vitro*, an activity which

Fig. 2 Restoration of antibody production by spleen cells from Nu/Nu mice with TNS. Cultures (in MEM + 5% FCS) were immunised with BRBC. Anti-BRBC (a) and anti-SRBC PFC (b) were assayed on day 4. △, TNS; ●, normal mouse serum; ○, serum from mice treated with BCG only; □, serum from mice treated with LPS only.

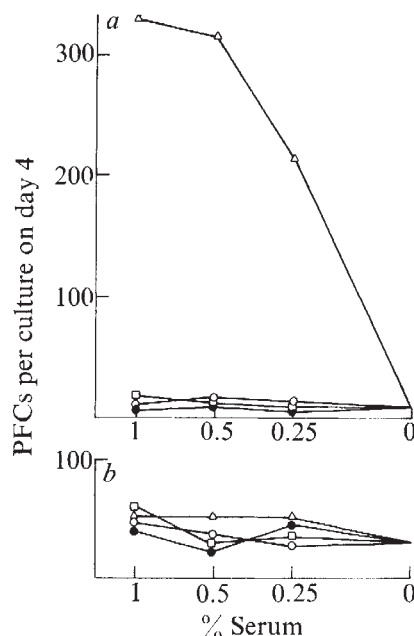


Table 1 Effects of TNS on production of antibody to SRBCs *in vitro*

Addition to culture*	Anti-SRBC PFCs per culture	
	Day 3	Day 4
None	65	3,900
TNS	1,850	13,500
TNF	1,280	10,200
Normal mouse serum	28	4,000
LPS serum†	108	6,500
BCG serum‡	78	4,000

*Final concentration of serum 2%, TNF 0.13 mg ml⁻¹.

†Serum from mice given LPS only.

‡Serum from mice given BCG only.

resides in the same serum fraction (TNF) as the tumour-necrotising activity.

We have already indicated that TNS does not substitute for that function of macrophages which can be replaced by 2-ME. We have described another function of macrophages, namely, the support of antibody formation *in vitro* by mouse spleen cells which are depleted of B cells carrying complement receptors (CR⁺)¹³. CR⁻ spleen cells are incapable of cooperating with primed helper cells in culture unless macrophages are present. In this context, macrophages cannot be replaced by 2-ME, but they can be replaced by culture supernatants of peritoneal macrophages that have been activated *in vivo* by injection of BCG. We have now found that TNS induces CR⁻ spleen cells to produce antibody to SRBCs and to cooperate with primed helper cells.

CR⁻ spleen cells were obtained by passage of mouse spleen cells through Sephadex G-10 columns coated with complement-reacted antigen-antibody complexes. This type of column removes macrophages and CR⁺ B cells¹³. As a control, spleen cells were passed through uncoated Sephadex G-10 columns, which remove macrophages¹¹, but not CR⁺ B cells¹³. CR⁻ spleen cells from mice primed with SRBC were used as a source of primed helper T cells¹⁴. One part primed cells (depleted of CR⁺ cells) was mixed with 9 parts unprimed CR⁻ cells, the cultures were sensitised with trinitrophenyl (TNP)-conjugated SRBCs, and the number of anti-TNP PFCs was determined on day 4. As shown in Fig. 3, CR⁻ spleen cells not only generated considerably less anti-TNP PFCs than CR⁺ spleen cells, but also failed to cooperate with SRBC-primed helper T cells. TNS induces CR⁻ spleen cells to produce antibody and to cooperate with primed helper T cells in the absence of macrophages. We have postulated¹³ that CR⁻ B cells must differentiate into CR⁺ B cells to carry out this function. This step in B-cell differentiation may be induced by macrophages when they are activated as, for example, after injection of BCG or by activated helper T cells¹³. As we will report elsewhere, TNF induces differentiation of CR⁻ B cells to CR⁺ B cells *in vitro* (M.H. and U. Hammerling, unpublished).

The cellular origin of TNF is not as yet known. For various

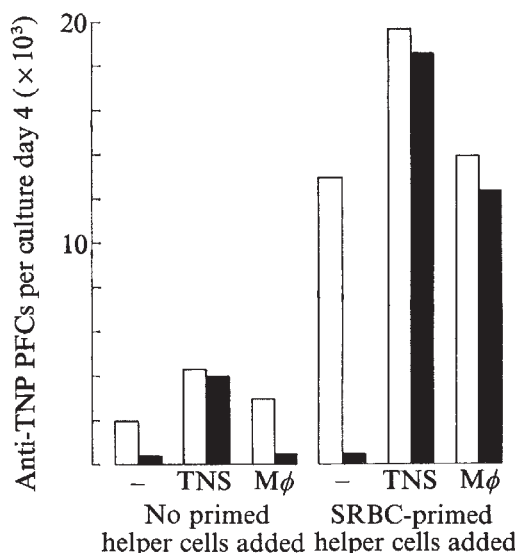


Fig. 3 Induction of antibody production in cultures of CR⁻ spleen cells by TNS. Spleen cells were passed through Sephadex G-10 columns coated with complement-reacted antigen-antibody complexes (CR⁻ spleen cells, hatched bars) or uncoated Sephadex G-10 columns (CR⁺ spleen cells, open bars). TNS was added at 1% final concentration. As source of macrophages (Mφ), 5 × 10⁴ peritoneal cells were added to the cultures. Average of 2 cultures

reasons, macrophages have been considered a likely source^{8,9}. The most striking phenomenon which occurs during the priming phase for TNF production is proliferation and activation of macrophages. Moreover, activated macrophages or culture supernatants of activated macrophages have been reported to possess activities that are similar to those of TNF. Like TNF they induce antibody formation in cultures of spleen cells from Nu/Nu mice, particularly after exposure to LPS. In addition, macrophages exposed to LPS become cytotoxic for transformed target cells in tissue culture^{15,16}, and TNF shares this property⁸. Some effects of TNF, on the other hand, mimic effects of factors assumed to be derived from T cells. As these T-cell factors have been reported to react with antisera directed against Ia and β₂ microglobulin⁵, use of such antisera may be useful for further definition of TNF. Until TNF has been fully characterised, however, it would be premature to ascribe the immunological effects reported here to the anti-tumour factor.

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Table 2 Substitution of T-cell helper function by simultaneous as well as delayed addition of TNS or TNF

Additions	Anti-SRBC PFCs per culture on day 4	
	(addition on day 0)	(addition on day 2)
None	<5	<5
TNS	170	390
TNF	230	310

Cultures of spleen cells of Nu/Nu mice were immunised with SRBCs on day 0 and assayed for anti-SRBC PFCs on day 4. TNS 1%, or TNF 0.13 mg ml⁻¹, were added as indicated on day 0 or day 2. Each value is the average of 3 cultures.

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Dissociation of alcohol tolerance and dependence

CHRONIC intoxication with ethanol has been shown to result in tolerance to and physical dependence on this drug^{1,2}. In general, the development of tolerance and physical dependence during intake of addictive compounds have been looked on as inexorably linked phenomena produced by a common biochemical³ or physiological⁴ mechanism. This was supported by the studies of Way *et al.*⁵ who demonstrated a simultaneous development and disappearance of tolerance and physical dependence after morphine pellet implantation in mice. We were interested in the possible application of the same theoretical framework to the development of tolerance to and physical dependence on ethanol. Previous work^{6,7} demonstrating changes in noradrenergic systems in brain after chronic treatment led us to study the effect of 6-hydroxydopamine (6-OHDA) pretreatment on the development of tolerance to and physical dependence on ethanol.

In our studies, one group of C57BL/6 male mice was injected intracerebroventricularly⁸ with 6-OHDA (50 µg in 10 µl artificial cerebrospinal fluid (CSF)⁹ containing 5 mg ml⁻¹ ascorbate), a second group of mice was injected with only the artificial CSF with ascorbate, and a third group of animals received artificial CSF containing no ascorbate. Since the presence of ascorbate in the artificial CSF had no effect on any of the parameters discussed below, all reported comparisons were between groups of animals injected with the CSF containing ascorbate and those injected with CSF, ascorbate and 6-OHDA. Brain levels of dopamine¹⁰, noradrenaline¹⁰ and 5-hydroxytryptamine (5-HT)¹¹ were determined in aliquots of two pooled brains^{8,12} seven days after the intracerebroventricular injection and then again after chronic ethanol treatment. These levels were 6.47 ± 0.87 , 1.47 ± 0.29 and 3.24 ± 0.48 nmol per g brain, respectively for the animals injected with 6-OHDA and 8.88 ± 1.60 , 3.26 ± 0.46 and 3.44 ± 0.29 nmol per g brain, respectively for those injected with artificial CSF. The levels of these neurotransmitters were not significantly altered by the chronic ethanol treatment described below.

A week after the injection, mice were placed in individual cages and offered a liquid diet consisting of Carnation Slender, sucrose (100 g l⁻¹) and Vitamin Diet Fortification Mixture (ICN Pharmaceuticals) (3 g l⁻¹) as their only food source. Twenty-four hours after being placed on the liquid diet, mice were divided into four groups:

Group 1: animals injected with artificial CSF. This group continued to receive the sucrose-containing diet throughout the period when the other animals (groups 3 and 4) received ethanol.

Group 2: mice injected with 6-OHDA and continued on the diet containing sucrose as were those in group 1.

Group 3: mice injected with artificial CSF. These mice were fed a diet in which the sucrose was substituted with 7% v/v ethanol.

Group 4: mice injected with 6-OHDA and fed the diet containing the 7% v/v ethanol as were those in group 3. Animals were maintained on their respective diets for seven full days, and on the morning of day 8, all animals again received the diet containing sucrose (withdrawal). During the period that mice received ethanol, body weight, behavioural intoxication, body (rectal) temperature, quantity of diet consumed and blood ethanol levels¹³ were monitored daily¹⁴. The quantity of diet given to the mice consuming the sucrose-containing mixture was made equal to the amount of ethanol-containing diet consumed by the animals in groups 3 and 4.

No differences were found in the daily amounts of diet consumed or blood ethanol levels during the chronic treatment with ethanol between animals in group 3 compared with those in group 4 (*t* test, $P > 0.25$). Behavioural intoxication scores¹⁴ were also not significantly different between these two groups

of animals. Body temperature was, however, found to be lower in animals of group 4 compared with those of group 3 (*t* test, $P < 0.05$) during the last two days on the ethanol diet. A more complete description of the animals during the period of chronic ethanol intake will appear elsewhere (Ritzmann and Tabakoff, in preparation).

After withdrawal from ethanol, animals were observed over a 12-h period: six 1-h periods of observation alternating with 1-h periods during which no observation took place. During the observation periods, body temperature was measured and behavioural signs of withdrawal were noted¹⁴. Withdrawal signs were scored as follows: 0 = no withdrawal symptoms; 1 = characteristic tremors and spasms when mice are lifted by the tail¹⁵; 2 = spontaneous tremors and spasms; 3 = spontaneous seizures (clonic and/or tonic)¹⁴; and 4 = death.

The behavioural withdrawal scores and measures of the concomitant hypothermia¹⁴ are summarised in Table 1. No significant differences (see legend to Table 1) in either the severity of the hypothermia or the behavioural manifestations of withdrawal were evident when animals from groups 3 and 4 were compared. Animals receiving the sucrose-containing

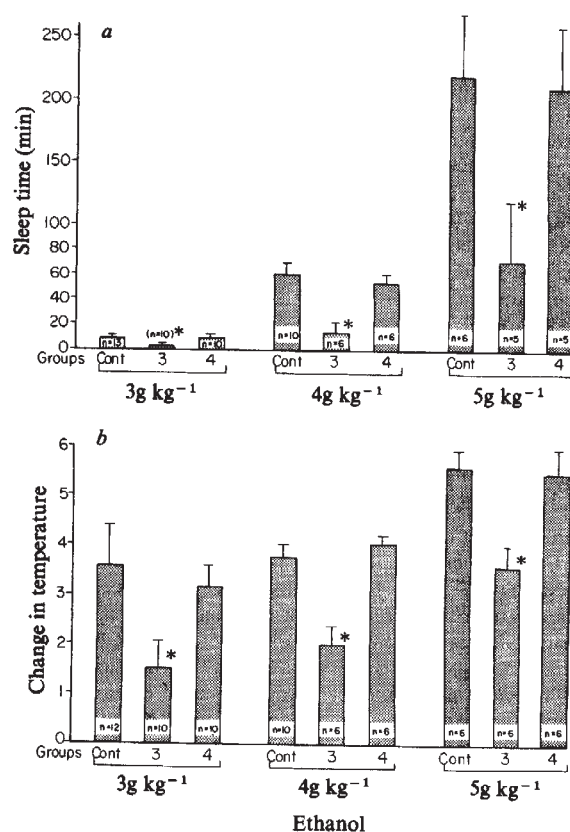


Fig. 1 Body temperature and sleep time after ethanol. Mice were injected intraperitoneally with ethanol (3, 4 and 5 g kg⁻¹) 26 h after withdrawal, and sleep time (a) and body temperature (b) were monitored. Since no differences in these parameters or blood ethanol levels were found between animals in groups 1 and 2, the values obtained with these animals were pooled and are labelled "control." Sleep time represents the time from loss of righting reflex to the time the mouse could right itself twice in 30 s. The bars represent the mean $\pm$ s.d., and the number within each bar is the number of animals used for the determination. Rectal temperature was monitored at 15- and 30-min intervals for 3 h after ethanol injection. The bars represent the mean $\pm$ s.d. of the greatest change from preinjection temperatures recorded during this period. The nadir for the 3 and 4 g kg⁻¹ dose occurred at 30 min and for the 5 g kg⁻¹ dose at 60 min after injection. Comparison between groups at each dose was made using the *t* test as well as the Mann-Whitney U test. *Groups significantly different from each of the other groups ($P < 0.05$) at a particular dose; other groups were not significantly ($P > 0.2$) different from one another.

Table 1 Hypothermia and withdrawal symptoms in mice

Behavioural withdrawal score	Time after withdrawal (h)					
	0	2	4	6	8	10
Group 3:						
0	36.2±0.4 (n = 10)	36.±0.4 (n = 9)	6 36.1±0.1 (n = 2)	—	36.9±0.2 (n = 4)	36.9±0.4 (n = 11)
1	—	35.0 (n = 1)	35.1±0.7 (n = 6)	35.8±0.6 (n = 6)	35.9±0.6 (n = 6)	36.0 (n = 1)
2	34.0 (n = 1)	34.2 (n = 1)	34.6±0.3 (n = 4)	34.2±0.7 (n = 6)	35.7±0.1 (n = 2)	—
3	33.0 (n = 1)	33.5 (n = 1)	—	—	—	—
4	—	—	—	—	—	—
Group 4:						
0	35.3±0.4 (n = 12)	36.5±0.5 (n = 11)	36.4 (n = 1)	36.4 (n = 1)	36.9±0.1 (n = 2)	36.6±0.5 (n = 8)
1	—	35.8 (n = 1)	35.1±0.7 (n = 6)	35.5±0.5 (n = 5)	36.3±0.9 (n = 4)	35.8±1.2 (n = 3)
2	—	—	34.3±0.6 (n = 4)	34.4±0.4 (n = 4)	35.1±0.9 (n = 5)	—
3	—	—	—	34.8 (n = 1)	—	—
4	—	—	—	—	—	—
	—	—	(n = 1)	—	—	—

Ethanol was removed from the diet of mice after 7 d of chronic consumption, and behaviour and body temperature were monitored. Mice receiving similar withdrawal severity scores at the various times of testing were grouped and the mean $\pm$ s.d. of the body temperature for each such group was determined and noted in the table. *n* represents both the number of animals exhibiting the particular withdrawal symptoms at the time of testing and the number of animals used to determine the mean temperature for the group. Hypothermia occurring in groups 3 and 4 was compared, after pooling the temperature values at each time point, using Student's *t* test. To assess whether or not behavioural withdrawal scores were different between groups 3 and 4, mice were subdivided as those receiving a score of 0 or 1 and those receiving a score of 2 or higher at each time point. Statistical analysis was performed using the Fischer Exact Probability test. No statistically significant differences were found between the body temperatures or withdrawal scores of groups 3 and 4.

control diet (groups 1 and 2) demonstrated none of the withdrawal symptoms. In separate experiments withdrawal hyperexcitability was assessed by injecting animals with pentylenetetrazol (60 mg kg⁻¹, intraperitoneally) and monitoring the incidence of convulsive seizures and death occurring within a 30-min period following such injection¹⁶. Pentylenetetrazol was administered to animals between 6 and 7 h after withdrawal of ethanol.

Animals which had been withdrawn from the ethanol-containing diets were significantly (binomial test¹⁷; *P* in all cases < 0.025) more susceptible to pentylenetetrazol-induced convulsions than sucrose-fed controls. No statistically significant differences (*P* > 0.3) in severity or frequency of pentylenetetrazol-induced seizures were, however, noted when comparing animals of group 3 with those of group 4. Eight animals from each of the four groups were tested, and the number of animals exhibiting clonic or tonic convulsions or dying in seizures after treatment with pentylenetetrazol in group 1 was two; in group 2, two; in group 3, six, and in group 4, seven.

Twenty-six hours after withdrawal, when all overt signs of the withdrawal syndrome were no longer present, mice were injected intraperitoneally with ethanol and "sleep time"¹⁸ and rectal temperature were monitored. Mice previously injected with pentylenetetrazol were not used in these studies.

Animals of group 3 were found to be tolerant to the effects of ethanol by measures of both rectal temperature and "sleep time" (Fig. 1). On the other hand, pretreatment of the animals with 6-OHDA before the chronic ethanol treatment (group 4) resulted in a block in development of tolerance to the measured effects of ethanol (Fig. 1). No significant differences in blood ethanol levels during the time that behavioural and physiological parameters were being measured were evident between any of the groups after injection of ethanol (3 g kg⁻¹).

Thus, animals treated with 6-OHDA and force fed a diet containing ethanol were found to become physically dependent on ethanol (as demonstrated by the appearance of withdrawal symptoms), but did not become tolerant to two of the major physiological effects of ethanol administration, that is, hypothermia and sedation.

Administration of 6-OHDA has been shown to result in the

destruction of catecholamine-containing neuronal terminals¹⁹. Such destruction of the functional connections between the noradrenergic neurones and other brain neurones seems to prevent the necessary alterations in neural cybernetics which produce tolerance after chronic ethanol treatment. Although the injections of 6-OHDA did not produce a statistically significant (*t* = 1.87, d.f. = 6) decrease in the levels of dopamine in our mice, the levels were substantially altered. Thus, we do not feel that the block in the development of tolerance can at present be totally ascribed to the destruction of only the noradrenergic systems.

The dissociation of tolerance and physical dependence witnessed in the present study provides an important conceptual framework within which the neurological effects of ethanol should be viewed. Seevers and Woods²⁰ and Kissin²¹ suggested previously that tolerance and physical dependence may not necessarily be unitary phenomena, and our studies demonstrate that certain manifestations of tolerance to ethanol may be eliminated without affecting the development of physical dependence produced by this drug.

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Progressive glomerulonephritis in mice treated with interferon preparations at birth

In addition to the well known antiviral action¹ interferon preparations exert various biological effects on cells (for references see refs 2 and 3). While investigating the effect of interferon on the growth and development of newborn mice, we found that daily injection of potent mouse interferon preparations resulted in extensive liver cell degeneration and death between days 8 and 14 (ref. 4). When interferon treatment, begun at birth, was stopped between days 6 and 8 of life, liver cell damage appeared to be reversible and most mice recovered. In the ensuing months, several of these mice died and although the liver and other organs appeared normal, the kidneys were pale and the surface was granular. Histological examination revealed a severe glomerulonephritis. We report here an investigation of this phenomenon.

Newborn Swiss (and in one experiment C3H mice) from specific pathogen-free colonies were injected subcutaneously in the interscapular region with 0.05 ml of mouse interferon or the different control preparations daily from day 0 to days 6-8, at which time treatment was stopped. When interferon-treated mice were killed on day 8, histological sections of liver showed focal cellular necrosis, as reported before⁴, but the kidneys appeared normal. Nevertheless, Swiss mice began to die with renal lesions as early as day 35. Autopsy of some mice dying with kidney disease showed signs suggestive of cardiac failure, that is enlarged heart, and haemorrhagic and oedematous lungs.

Mice were killed at regular intervals, and sections of organs were examined under light microscopy. In all cases, the kidneys of Swiss mice treated with interferon at birth showed glomerulonephritis (Table 1) whereas the liver appeared normal. The kidney pathology can be summarised as follows. In "early" lesions (Swiss mice killed on the 20th day of life, Table 1) the glomeruli appeared moderately enlarged with some thickening of the mesangium, some segmental foci of mesangial proliferation and some thickening of peripheral capillary loops. There were no tubulo-interstitial lesions except for a few mononuclear cells. The vessels appeared normal. By immunofluorescence there were focal and segmental granular deposits of IgG, IgM and C3 in the mesangial spaces and along some glomerular basement membranes (GBM). Fibrinogen and IgA were not detected. In Swiss mice killed after 31 d, the lesions were more conspicuous with hyalinisation of glomerular tufts (with epithelial crescents and voluminous subendothelial deposits) (Fig. 1a). In well advanced disease, virtually all glomeruli were sclerotic. Amyloid was not detected. The tubulo-interstitial lesions paralleled the glomerular damage, showing extensive sclerosis, and diffuse atrophy of tubules with dilated lumina filled with hyalin and proteinaceous casts (Fig. 1b). By immuno-

Table 1 Appearance of glomerulonephritis in mice treated for 6-8 d from birth with mouse interferon preparations

Strain of mouse	No. of experiments	Age of mice killed (d)	Mouse* interferon	Control preparation	Inactivated† mouse interferon	Human‡ interferon	Uninoculated
Swiss	9	20	4/4 (1.2)				0/2
		31-35	10/10 (1.9)	0/3	0/3	0/3	0/5
		45-60	7/7 (3.3)	0/9			0/7
		67-82	12/12 (3.3)	0/2	0/4	0/4	0/5
		85-100	8/8 (3.4)	0/1			0/5
		102-124	10/10 (2.8)	0/8	0/5	0/5	0/11
		143-185	9/9 (2.4)	0/5	0/6	1/6¶	0/7
		198-274		0/22			2/23
	Total		60/60	0/50	0/18	1/18	2/65
C3H	1	43-55	8/8 (2.0)				0/5
		104-132	5/9 (0.6)				0/8
	Total		13/17				0/13

Mouse interferon was prepared from Swiss mouse C-243 cells induced with Newcastle disease virus (NDV), and concentrated, partially purified and assayed as described before⁵. Mock interferon was prepared in a manner identical to that used in the preparation of interferon with the exception that the interferon inducer, NDV, was either omitted, or added for 1 h immediately before collection of the culture supernatant. All interferon and control preparations were dialysed further for 24 h at 4°C against 0.01 M perchloric acid before testing for toxicity on a line of L 1210 cells resistant to interferon⁶. The specific activity of mouse C-243 cell interferon preparations was in the range $0.7-3.0 \times 10^6$ U per mg protein. In one experiment mice were treated with a more purified C-243 interferon preparation (1.9×10^7 interferon reference U per mg protein) (purified by affinity chromatography using sheep anti-mouse interferon globulins). C-243 cell control preparations contained approximately the same amount of protein per ml as the C-243 cell interferon (that is $1.5-3.4$ mg ml⁻¹). Partially purified human interferon (specific activity 3.5×10^5 U per mg of protein) prepared in leukocyte suspensions inoculated with Sendai virus was provided by Dr K. Cantell⁷. Mouse interferon units quoted are as measured in our laboratory and one of our units equals 4 mouse interferon reference units.

* A different mouse C-243 cell interferon preparation (titre 1:800,000 to 1:1,600,000) was used in each experiment. Only mice having been injected daily from birth to days 6 to 8 of life are included.

† Mouse C-243 interferon with a titre of 1:800,000 was heated at 60°C for 2 h at pH 10. Residual titre was 1:20.

‡ Titre of human leukocyte interferon was 1:1,000,000 (MRC reference interferon 69/19 U) as assayed on human diploid fibroblasts.

§ No. of mice with histological confirmation of glomerulonephritis per total number of mice killed. Each kidney was examined by light microscopy and the severity of lesions was graded on a scale from 0 to 4. In all cases there was little variation in the extent of lesions within a given series and the figures in brackets indicate the mean index of severity of kidney lesions for each series.

¶ The kidney of one mouse showed glomerulonephritis grade 2.

|| The kidneys of two mice showed glomerulonephritis grade 1.

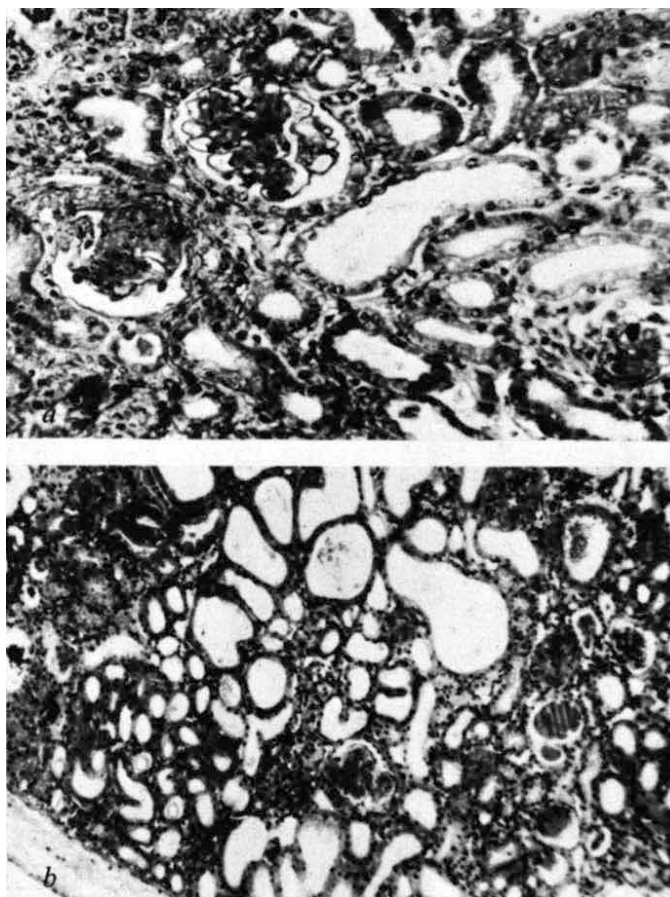
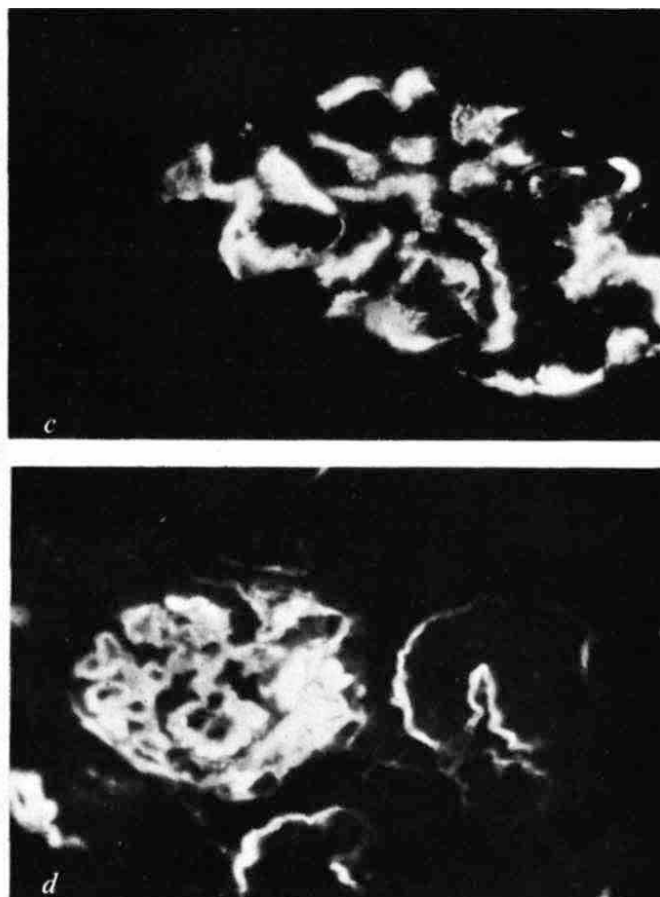


Fig. 1 Kidneys of Swiss mice treated with mouse interferon for the first 7–8 d of life. *a*, Mouse killed at 100 d. Note mild glomerular proliferation and hyalinisation of glomerular tufts. Tubular epithelium appears flattened with dilatation of lumina. There are a few inflammatory cells in the interstitium ($\times 250$). *b*, Mouse killed at 60 d. Note extensive sclerosis of interstitial tissue (with cellular infiltration) with pronounced atrophy of tubules and sclerotic glomeruli ($\times 100$). *c*, Mouse killed at 85 d. Stained with fluoresceinated anti-mouse IgG serum. Note coarse granular deposits along GBM ($\times 540$). *d*, Stained with fluoresceinated anti-mouse C3 serum. Note deposits of C3 along GBM. The deposits of C3 along the tubular basement



membrane have no pathological significance as they are also present in control mice ($\times 400$). Renal tissue was fixed in Dubosq–Brazil fixative (alcoholic Bouin) for 2 d and then in 4% formalin in saline, and cut at 3 μ m. The following stains were used systematically: Mallory's trichomic, periodic acid-Schiff, haematoxylin and eosin, Weigert's fuchsin, Wilder's reticulin and crystal violet. Immunofluorescence was done on snap-frozen specimens cut in a cryostat. Rabbit anti-mouse IgG, IgA, IgM, C3 and fibrinogen sera were obtained from Cappel Laboratories. The specificity of each antiserum was checked by immunoelectrophoresis. All magnifications are original magnifications.

fluorescence there were granular coarse deposits of IgG, IgM and C3 along the GBM (Fig. 1c and d). Fibrinogen was detected in crescents. IgA was not detected.

The results of our experiments can be summarised as follows. (1) In nine experiments, all Swiss (60 out of 60) mice treated with potent interferon preparations for the first 6–8 d of life developed severe glomerulonephritis early in life (Table 1). Only one out of 86 mice treated with the various control preparations and two out of 65 untreated mice developed a moderate glomerulonephritis and this occurred only in mice kept for more than 143 d (Table 1). (The finding of glomerulonephritis in some ageing Swiss mice is in accord with the results of Oldstone and Dixon who reported a "mild nephritis seen in 7% of SWR/J control mice by 9 months"⁸.)

(2) Glomerulonephritis developed only in mice inoculated with enough interferon and for a sufficient time. Thus, only mice inoculated with interferon preparations titring approximately $1:10^6$ for 6–8 d developed glomerulonephritis. Mice injected for 8 d with interferon preparations of lower titre, that is $1:10^5$ (23 mice), or $1:10^4$ (18 mice), showed no signs of glomerulonephritis when killed at 85, 140 and 198 d. In addition, injection of potent interferon preparations ($1:10^6$) did not result in glomerulonephritis when injected for only the first 2 d of life (20 mice killed at 85, 140 and 198 d).

(3) Interferon treatment resulted in glomerulonephritis only when administered during the first week of life. Thus,

none of nine mice inoculated between the 8th and 15th d of life with an interferon preparation titring $1:10^6$ showed glomerulonephritis when killed at day 59. (4) Male and female mice seemed to be equally susceptible as judged by the severity of disease and the incidence of death.

(5) The progression of the disease in Swiss mice was observed by killing mice at intervals and by following the progression of lesions in the same mouse after unilateral nephrectomy. Kidney lesions were minimal at 3 weeks, definite at 1 month and considerably more advanced in the ensuing weeks. (Table 1: note figures in brackets indicating mean index of severity of kidney lesions graded on a scale from 0 to 4). A kidney from each of two interferon-treated mice was removed on day 31 and the mice were killed on day 45 and 60. In both cases a progression of lesions in the remaining kidney was observed. (Unilateral nephrectomy in control mice did not cause glomerulonephritis in the contralateral kidney.) Since a number of interferon-treated mice died (probably of renal failure) after 4 weeks of life, it is possible that the apparent decrease in the severity of kidney lesions in mice older than 102 d (Table 1) reflects the selection of surviving mice with less severe disease rather than a reversibility of the pathological process.

(6) Although glomerulonephritis also developed in C3H mice treated from birth for 6–8 d with mouse interferon preparations titring $1:10^6$ (according to the protocol used for Swiss mice) (Table 1), the glomerular lesions appeared

to be less severe than in Swiss mice at the same age, and deaths were not observed. Kidneys of eight out of eight C3H mice killed at 43–55 d showed moderate glomerulonephritis (Table 1). At 104–132 d, although glomerular lesions were present in six out of nine mice, these were less marked than in mice killed earlier. Furthermore, a kidney biopsy, at 45 d, of an interferon-treated C3H mouse showed moderate disease, whereas only minimal lesions were present when this mouse was killed at 104 d. Thus the glomerulonephritis in C3H mice is not only less severe than in Swiss mice, but may be reversible. (It is interesting in this regard that C3H mice developed a less severe glomerulonephritis than SWR/J mice when infected transplacentally or neonatally with lymphocytic choriomeningitis virus⁸.)

Because there was a lapse of several weeks between cessation of interferon treatment (days 6–8) and the appearance of glomerulonephritis (day 20), and as interferon is cleared rapidly from the circulation^{10–13}, it seems unlikely that the development of progressive nephritis was due to a local retention of interferon. It is possible, however, that interferon damaged the kidney during the first week of life directly, or indirectly perhaps through its toxic effect on the liver^{4,14}. Alternatively, this treatment might have altered the maturation of the kidney or some other organ(s), such as the spleen⁴, leading ultimately to the development of glomerulonephritis.

The rapidity of onset of the glomerulonephritis and the histological lesions themselves do not permit ready classification in terms of pathogenesis^{18–19}. The absence of linear deposits of Ig argues against the hypothesis of an anti-GBM glomerulonephritis and anti-GBM antibodies were not present in the sera of sick mice tested by indirect immunofluorescence. The granular pattern of the Ig and C3 deposits seem to favour the hypothesis of an immune complex nephritis, although we have no idea about the nature of a hypothetical antigen. We have never detected neutralising antibody to interferon in either the serum or γ -globulin-containing eluates of the kidneys of interferon-treated mice (neither was interferon itself present in these eluates), nor is there any evidence to suggest that interferon treatment during the first week of life activates a latent virus⁴. Anti-DNA antibodies were also not detected in the serum of sick mice.

Several chronic virus infections in animals can result in the late development of nephritis which is associated with the progressive deposition of virus-antibody complexes in the glomerulus^{20–22}. Perhaps some of these viruses induce interferon in the neonatal period, contributing to the further development of glomerulonephritis. Furthermore, if our results in mice can be extrapolated to man, they suggest that an acute virus infection of the human newborn, if associated with the production of sufficient interferon, might result later in life in the development of a progressive glomerulonephritis.

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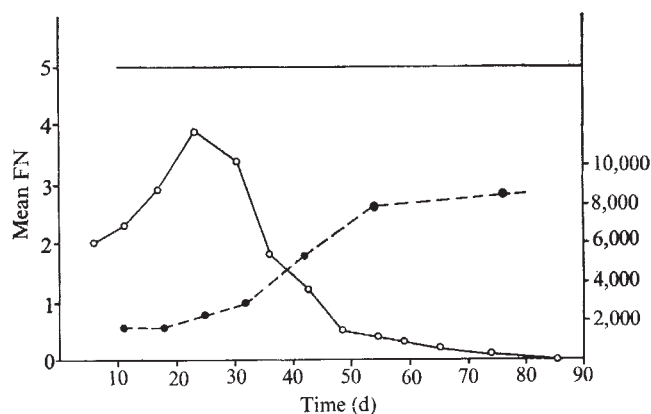
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Postponement of symptoms of hereditary muscular dystrophy in chickens by 5-hydroxytryptamine antagonists

THE commoner forms of muscular dystrophy, both in man and in animals, are determined by a single gene change, but the mechanism whereby the complex set of symptoms develops is unknown. Chickens homozygous for muscular dystrophy have been bred, and form probably the closest animal model for the human disease so far investigated^{1,2}. Dystrophic line 304 of the Davis (California) flock, for example, has been bred for rapid appearance of the symptoms and shows some signs of muscle weakness at 7–10 d *ex ovo*, whereas a normal line (200) of chickens with closely related genetic background is also available^{1,2}. Two of us have reviewed³ evidence that 5-hydroxytryptamine (5-HT) is involved in some way in the development of the symptoms of muscular dystrophy, and reported that administration of a 5-HT antagonist, methysergide, retards the development of the symptoms in line 304 chickens. We report here that this effect is more general, in that a second anti-serotonergic drug of entirely different chemical struc-

Fig. 1 Mean FN (○) and plasma CPK level⁸ (●) for untreated dystrophic chickens, as a function of age (50–82 animals were used per point). The horizontal line shows the constant FN for the normal chickens from day 10 onwards.



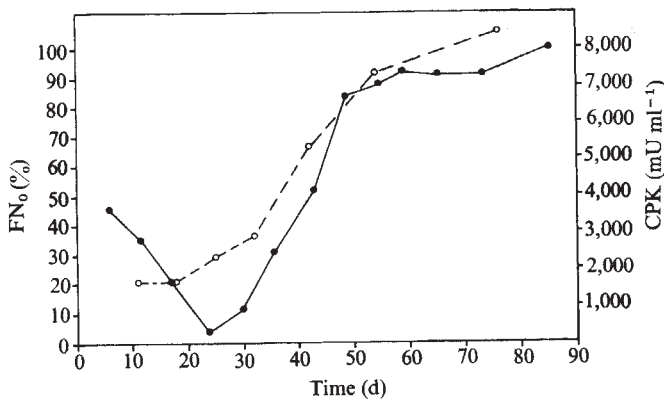


Fig. 2 Percentage of the untreated dystrophic population (50–82 animals per point) giving FN = 0 (●), and the corresponding plasma CPK level (○).

ture, cyproheptadine, is also effective, and that a combination of the two agents is beneficial.

We first established that the development of measurable symptoms in line 304 dystrophic chickens is regular enough for any drug-induced improvement to be reliably detected. A test^{1,4,5} of muscle weakness in dystrophic chickens was used in the form of the 'flip number' (FN)—that is, the number of times (0–5 out of 5) a bird can right itself when placed on its back five times in immediate succession. The considerable increase in the creatine phosphokinase (CPK) content of the blood plasma, known to characterise this disease in the chicken⁶ and in man⁷, was used as a second criterion of muscle damage. Using a total population of 150 dystrophic chickens (line 304, Department of Avian Sciences, University of California, Davis) maintained in groups from different hatches over a 2-yr period, these two parameters were followed from hatching for 80 d. Chickens were maintained in constant conditions (temperature 30–33 °C, 12 h light/dark cycle), and the flip test was given in a precisely standardised manner by a naive observer, at 5-d intervals to avoid an exercise component. CPK testing was carried out in a completely blind fashion on coded sera in another laboratory. Newly hatched chicks of the normal line do not develop sufficient muscle strength to give FN=5/5 until 7–10 d of age and show high variability until then, but thereafter are constant, all at 5/5 (Fig. 1). The dystrophic chicks improve partially until about 3 weeks of age, and thereafter show a sharp decline to low scores, eventually zero. The plasma CPK level increases by about 400% throughout the latter period (Fig. 1). We have found that a very good correlation between the muscle weakness and release of CPK from the muscle can be demonstrated (Fig. 2) using as the measure of the former the FN₀ value—that is, the percentage of the dystrophic population that fails totally (having FN=0/5).

With this baseline for comparison, birds injected³ (twice

Table 1 FN differences in treated and untreated chickens

	10–13	Days <i>ex ovo</i> 41–44	56–60
Untreated	2.3±0.3 (57)	1.2±0.2 (56)	0.3±0.2 (56)
Methysergide	3.3±0.6 (15) [12.5]	5.0±0.0 (11)* [41]	4.0±0.7 (10)* [51]
Cyproheptadine	3.0±0.4 (9)	4.8±0.2 (9)*	1.7±0.8 (9)**
Methysergide + cyproheptadine		5.0±0.0 (27)*	4.6±0.1 (27)*

Values represent mean FN±s.e.m. for dystrophic chickens, with the number of animals used in parentheses. For the progressive dosages of cyproheptadine (medium dose) and the combination, see Fig. 3; for methysergide the dose level at each period is given in square brackets (in mg kg⁻¹ d⁻¹). Significance (using *t* test) from untreated dystrophic group: **P* < 0.001; ***P* < 0.01.

daily, intraperitoneally) with cyproheptadine hydrochloride (Periactin, donated by Merck, Sharp and Dohme, Rahway, New Jersey) from day 3 *ex ovo*, were tested. Untreated animals were given water injections similarly throughout. At a low dose regime (for the chicken), substantial improvement in the FN value was shown (Fig. 3). A medium level dose (12 mg kg⁻¹ d⁻¹) extended the later period of this effect, but the performance still declined sharply after day 55. The latter decline was further postponed by a combination of higher doses of cyproheptadine and methysergide (Fig. 3). For the latter case, all the treated animals remained at a passing level (FN=4–5) up to day 60, whereas all of the untreated birds were below this level from about day 30. Similarly, the FN₀ values for animals treated with cypro-

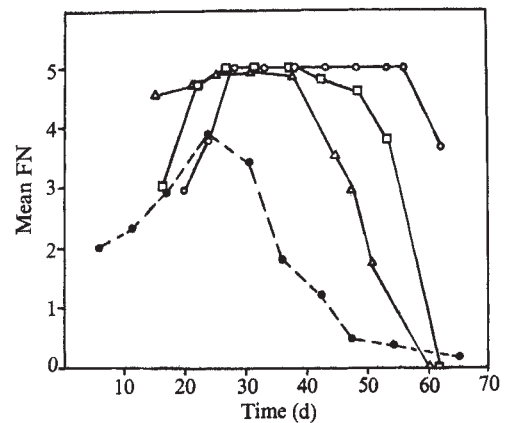


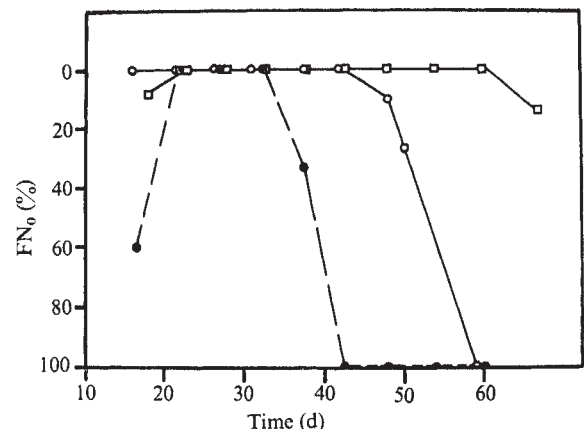
Fig. 3 Mean FN of cyproheptadine-treated or untreated (●), (see Fig. 1) dystrophic chickens. Low dosage regime (Δ, *n* = 12) was 2.4 mg kg⁻¹ d⁻¹ at days 3–16, 6.0 at days 17–43 and 8.0 at day 44 onwards. Medium dosage (□, *n* = 9) was 12.0 mg kg⁻¹ d⁻¹ from day 3 onwards. A combined treatment (○, *n* = 27) was also given, with cyproheptadine at 8.0 mg kg⁻¹ d⁻¹ at days 3–10, 12 at days 11–30, 17.5 at days 31–52, and 23 at day 53 onwards, plus methysergide at 12.5 mg kg⁻¹ d⁻¹ at days 3–15, 20 at days 16–30, 41 at days 31–45, and 52 at day 45 onwards.

heptadine or with the combination showed a pronounced difference from the untreated dystrophics (Fig. 4).

Using the criterion of CPK release, cyproheptadine-treated animals also showed a smaller change than the untreated birds (Fig. 5). The tendency to stabilise at a plateau of about 2,500 mU CPK per ml plasma in the treated birds, compared with about 8,500 in the untreated birds, is seen more clearly for the treatment with the two drugs combined.

Statistical data for points where divergences were

Fig. 4 Percentage of the population of dystrophic chickens having FN = 0, when untreated (●, *n* = 15), or treated with cyproheptadine (○, dose low or medium, see Fig. 3; *n* = 21) or treated with both drugs (□, doses as stated for Fig. 3; *n* = 27, or, for last point only, 15).



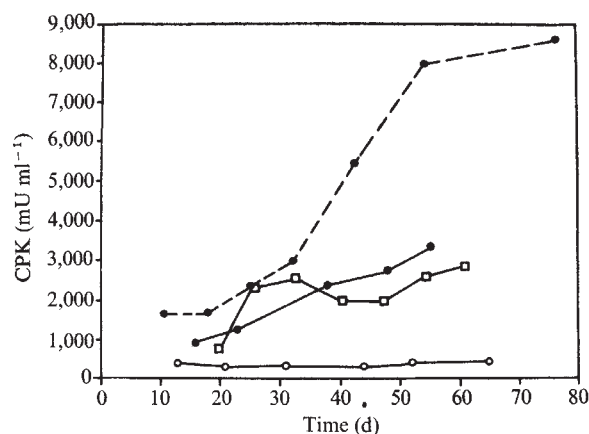


Fig. 5 Plasma CPK content of dystrophic chickens, untreated (broken line, mean of 18 birds per point), or treated with cyproheptadine (with FN = 5; ●, mean of 8 per point), or with both drugs (with FN = 5; □, mean of 9 per point). ○, Normal chickens (mean of 14 per point).

apparent are illustrated in Table 1. Standard errors typical of the CPK determinations have been given elsewhere³. For all cases a highly significant difference was found between the untreated and the treated groups, with cyproheptadine over days 30–55, and with the combination of drugs from day 30 to the end of the study.

The two drugs used are already administered chronically in man for other conditions. In the mouse⁹ LD₅₀ for toxicity of cyproheptadine given intraperitoneally is 55 mg kg⁻¹. In the chicken, it seems to be of still lower toxicity, perhaps denoting a more rapid removal or metabolism, since we have been unable to observe toxic symptoms at higher doses than that. We will report elsewhere more details of the conduct of the therapeutic trials, of histological evidence that parallels the other criteria, and of the lack of any effect in the dystrophic birds of these drug treatments on plasma enzymes unrelated to muscle disease.

There has been a long standing debate on the possibility of a change in blood circulation or of anoxia as a component in dystrophy (see refs 10 and 11). Our results may be attributable to antagonism of a 5-HT-induced effect on the microvasculature of dystrophic muscle, if such a component is indeed significant in inherited dystrophy; but that assumption is not as yet justified since the amelioration could, alternatively, arise by antagonism of an action of 5-HT elsewhere—for example, directly on the muscle¹². In either case, the results suggest that an investigation in these chickens of a range of 5-HT antagonists could be of therapeutic interest, and they may provide a clue to one of the determinants of the dystrophic process.

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Altered erythrocyte membrane phosphorylation in sickle cell disease

FOR several years evidence has been accumulating to indicate that the erythrocytes of individuals with sickle cell anaemia not only contain an abnormal haemoglobin but also exhibit several altered membrane characteristics. These include increased cation transport¹, possible alterations in phospholipid arrangement², and increased membrane rigidity³. The protein and glycoprotein content of membranes from erythrocytes of individuals with sickle cell anaemia does not seem to differ from that of normal erythrocyte membranes⁴. Many of the altered membrane characteristics appear to be a function of altered erythrocyte membrane structure due to membrane-haemoglobin interactions⁵, however, whether or not other membranous protein-protein interactions are altered is not well documented.

We have recently reported⁶ that human erythrocyte membranes contain, as well as a cyclic AMP-dependent protein kinase, another protein kinase which is capable of using either ATP or GTP as its phosphoryl donor. This enzyme is insensitive to regulation by cyclic nucleotides and catalyses the phosphorylation of membrane polypeptides in the area of bands 2 and 3 (nomenclature of Steck⁷). In this communication we report that the autophosphorylation of sickle-cell (SS) erythrocyte membranes by this enzyme differs from that observed with normal cells.

Blood was obtained from non-hospitalised individuals homozygous for haemoglobin S and from normal individuals through the Hematology Clinic at the University of Illinois

Fig. 1 Autophosphorylation of normal (a) and sickle cell (b) erythrocyte membranes in the presence of γ -³²P-GTP. Normal (38 μ g) and sickle-cell (34 μ g) erythrocyte membranes were incubated for 30 min at 37 °C in the presence of 0.1 M glycine-NaOH (pH 8.5), 10 mM MgCl₂, 0.2 mM γ -³²P-GTP (280 c.p.m. pmol⁻¹). The phosphopeptides were separated by electrophoresis in a 5% polyacrylamide slab gel containing 0.2% SDS. The figure represents a densitometric tracing of an autoradiogram which was obtained after a 3-d exposure to the dried gel. Other experimental details were as previously described⁶.

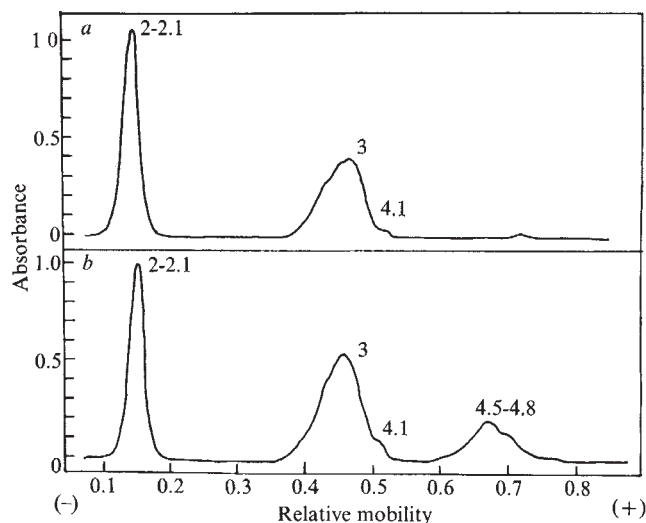


Table 1 Phosphorylation of normal and sickle-cell erythrocyte membrane polypeptides

Phosphoryl donor		2-2.1	3	4.1	4.5-4.8
ATP	Normal (5)	1.01±0.05	1.43±0.11	0.05±0.01	0.12±0.02
	SS (5)	0.74±0.05*	1.51±0.13	0.12±0.03	0.24±0.02
ATP (+ cyclic AMP)	Normal (5)	1.27±0.11	1.61±0.13	0.16±0.02	0.43±0.05
	SS (4)	0.94±0.07	1.48±0.23	0.22±0.06	0.58±0.13
GTP	Normal (7)	0.64±0.05	0.57±0.13	0.01±0.01	0.01±0.01
	SS (8)	0.51±0.03†	0.83±0.12	0.07±0.04	0.20±0.03‡

Membranes were phosphorylated exactly as described in Fig. 1 except that γ - ^{32}P -ATP (0.2 mM, 250 c.p.m. pmol⁻¹) and cyclic AMP (2 μM) were present as indicated. When ATP was used as the phosphoryl donor, the incubation period was 5 min at 37 °C and the autoradiogram was exposed for 4 d. The values were obtained by integrating the areas under the indicated peaks of densitometric tracings and are expressed as arbitrary densitometric units per μg membrane protein. All values are given as means $\pm$ s.e.m. The numbers in parentheses refer to the number of determinations. Statistical determinations were performed using the Student's *t* test.

* Significantly different from normal, $P < 0.01$.

† Significantly different from normal, $P < 0.05$.

‡ Significantly different from normal, $P < 0.001$.

Hospital. Haemoglobin-free erythrocyte membranes were prepared according to Dodge *et al.*⁸ and stored in liquid nitrogen before use. Membranes were phosphorylated and applied to 5% polyacrylamide slab gels containing 0.2% SDS as previously described⁶. Autoradiograms which were prepared from the dried gels were scanned using a Zeineh Soft-Laser densitometer.

In confirmation of a previous study by others⁴, we saw no large differences in the protein content of normal and SS erythrocyte membranes as analysed by SDS-polyacrylamide gel electrophoresis. When we studied the phosphorylation profile of the two types of membranes, however, we saw marked differences when γ - ^{32}P -GTP was used as the phosphoryl donor (Fig. 1 and Table 1). As mentioned above, only one of the membrane-bound kinases can utilise GTP as its phosphoryl donor. As seen in Fig. 1 and Table 1, one or more polypeptides which migrate in the area designated as 4.5-4.8 are substrates for the GTP-utilising kinases in sickle-cell but not in normal erythrocyte membranes. This difference is not observed when ATP is used as the phosphoryl donor, either in the presence or absence of cyclic AMP (Table 1).

A second, albeit less marked, difference in sickle-cell membrane phosphorylation is that the phosphorylation of polypeptides 2-2.1 is slightly lower in sickle-cell membranes than in normal erythrocytes when either ATP (in the absence of cyclic AMP) or GTP is used as the phosphoryl donor. Although there is a tendency for the phosphorylation of 2-2.1 in the presence of ATP and cyclic AMP to be less in the SS than in the normal erythrocyte membranes, the data are not significantly different. In the presence of cyclic AMP no significant differences in the phosphorylation of normal and SS membranes are observed.

There are several possible explanations for the observed results. We favour the hypothesis that the conformation of kinase substrates may be altered in the SS membranes. It is conceivable that phosphoryl acceptor sites are covered (as for 2-2.1) or uncovered (as for 4.5-4.8) as a result of repeated sickling and unsickling during the lifespan of the erythrocytes. Alternately, the kinase itself may be altered such that its substrate specificity is decreased. Since the cyclic AMP-dependent protein kinases do not use GTP as a phosphoryl donor⁹, it is unlikely that the phosphorylation of 4.5-4.8 in SS erythrocyte membranes in the presence of GTP is attributable to the cyclic AMP-dependent kinase. Further characterisation of the kinetics of the phosphorylation reactions in normal and SS membranes and the solubilisation of the kinases and their substrates is necessary to determine if one or more of these or other factors are responsible for the observed phenomenon.

It is well documented that several aspects of cation content and transport are altered in SS erythrocytes¹. In addition, there are several lines of evidence implicating membrane phosphorylation in the regulation of cation

transport^{10,11}. Whether or not the altered membrane phosphorylation of SS erythrocytes plays a role in the altered transport processes in these cells or in other membrane phenomena remains to be determined.

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Inhibition of intercellular adhesion in a cellular slime mould by univalent antibody against a cell-surface lectin

THE cellular slime moulds, *Dictyostelium discoideum* and *Polysphondylium pallidum* contain carbohydrate-binding proteins (lectins) that increase significantly in amount in soluble crude extracts as amoebae differentiate from the vegetative stage (when the cells do not associate) to the aggregation-competent stage (when the cells can form stable intercellular contacts)¹⁻³. Lectins from the two species, assayed as agglutinins of erythrocytes, have been purified by affinity techniques and, on the basis of their carbohydrate-binding specificities, as well as several physicochemical properties, have been shown to be distinct proteins³⁻⁵. Distinct lectin activities have also been identified in four other species of slime moulds⁶. The lectins from *D. discoideum* and *P. pallidum* accumulate on the cell surface with development of aggregation competence as demonstrated by erythrocyte-rosette experiments^{1,2,7}, immunofluorescent and immunoferritin techniques^{7,8}, and lactoperoxidase-catalysed iodination of intact cells⁹. Both species also contain cell-

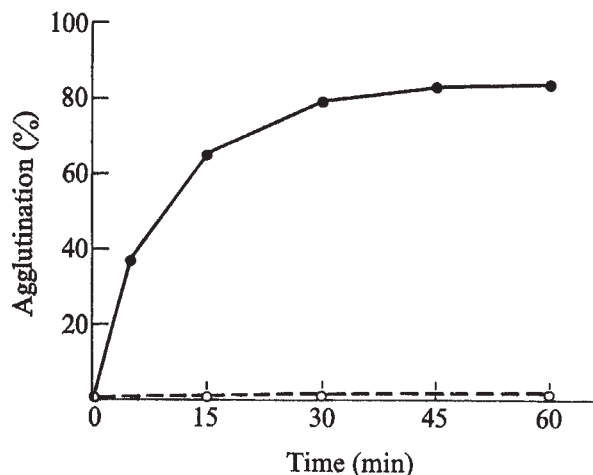


Fig. 1 Kinetics of agglutination of aggregation-competent amoebae (●) and vegetative amoebae (○). Vegetative amoebae and aggregation-competent amoebae were collected after 55 h and 96 h of incubation on agar, respectively. Cells were washed several times in cold water to separate them from bacteria and were then suspended at 9×10^6 ml⁻¹ in EDTA-phosphate buffer (16.7 mM Na₂HPO₄-KH₂PO₄, 10 mM EDTA, pH 6.2) containing 0.2 M D-glucose and 1 mg ml⁻¹ bovine serum albumin (BSA). The cell suspension was added to each of several individually cut out wells of a Linbro plate (16 mm diameter, FB-54). The wells were gyrated at 115 r.p.m. on a New Brunswick G-24 shaker (radius of gyration 3/4 inch) at 22 °C. At various times, the contents of wells were carefully poured into plastic vials containing 20 ml of EDTA-phosphate buffer. The vials were gently mixed and after 5 min, to allow osmotic re-equilibration, single cells were counted with a Coulter Counter (Model ZBI, 100-μm aperture, 1/amplitude = 1, 1/aperture current = 0.354, impedance = 10 K, threshold = 10-60). With these settings, approximately 90% of the single cells were counted. The total number of cells present in the suspension was determined by dispersing the agglutinates into single cells by Manostat pipetting and counting at the above settings. The percentage agglutination was computed as the percentage of single cells that entered into aggregates. Two wells were used for each time point. Readings on independent samples agreed to within 5%.

surface receptors with a very high affinity for the homotypic lectin and a lesser affinity for the heterotypic lectin¹⁰, suggesting a possible basis for species-specific intercellular affinities. In the case of *D. discoideum*, we have shown that the high affinity receptor appears on the cell surface as amoebae differentiate into the cohesive state¹⁰.

This series of findings raises the possibility that intercellular adhesion in the cellular slime moulds is mediated by the association of cell-surface lectins with complementary surface receptors, presumed to contain complex oligosaccharides. This possibility is supported by the finding that cohesiveness of aggregation-competent *P. pallidum* cells is inhibited by specific simple sugars that react with pallidin, the lectin derived from this species². High concentrations of these sugars are, however, required (0.1 M or higher), suggesting the possibility of nonspecific effects on unknown components of the cell, rather than inhibition of cell-surface pallidin. To test the possibility that pallidin on the surface of *P. pallidum* amoebae is indeed involved in the developmentally regulated cohesiveness of these cells, we raised antiserum to purified pallidin and determined the effect of Fab fragments, derived from the antibodies, on the cohesiveness of *P. pallidum* cells. We now report that these univalent antibodies inhibit the cohesiveness of differentiated amoebae.

Polysphondylium pallidum strain WS 320 was grown in the dark in the presence of *Aerobacter aerogenes* on

nutrient agar at 22 °C with 10^5 spores inoculated on each 100-mm agar plate, as described previously². In these conditions, the amoebae became aggregation competent by 96 h when the bacteria had been substantially cleared. The absence of light prevents the cells from aggregating and proceeding through culmination. On illumination, however, aggregation of the cells is apparent within 30 min. Vegetative cells were obtained after 55 h of culturing, when a thick carpet of bacteria still covered the agar. These cells, once free of bacteria, require a several-hour interphase period before they are aggregation competent. Cells of both stages were collected and washed as previously described². An index of cell cohesiveness was obtained by gyrating a suspension of mechanically dispersed cells and determining the percentage reduction in single cells as they agglutinated. In the hypertonic assay conditions used (see Fig. 1), only the aggregation-competent cells formed agglutinates, whereas vegetative amoebae remained as single cells. The critical factors in this assay, including a morphological study of the agglutinates by transmission and scanning electron microscopy, will be presented later.

Pallidin was purified by affinity adsorption³. The purified protein gave a single band on polyacrylamide electrophoresis in the presence of sodium dodecyl sulphate and 2-mercaptoethanol, even when as much as 200 μg of protein was applied to the gel. Antiserum, raised in a rabbit using a conventional procedure as described previously⁷, formed a single precipitin band on immunodiffusion against either

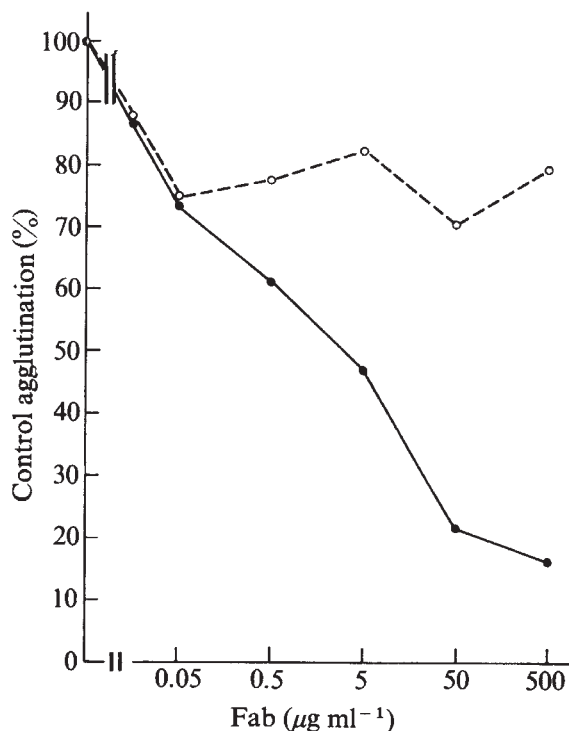


Fig. 2 Effects of immune (●) and normal Fab (○) on *P. pallidum* agglutination. Cells were collected after 96 h of incubation, washed and suspended to 1.8×10^7 ml⁻¹ in EDTA-phosphate buffer containing 0.4 M D-glucose and 2 mg ml⁻¹ BSA. Aliquots of the cells were dispersed and mixed with equal volumes of varying concentrations of normal or immune Fab diluted in EDTA-phosphate buffer. After a preincubation period of 30 min at 0 °C, the cells were dispersed again and added to Linbro plastic wells, 500 μl per well. After 30 min of gyration by which time equilibrium was approached, the percentage of cells that entered into agglutinates was determined as above (see Fig. 1 legend). For each of a series of concentrations of normal and immune Fab, the percentage of the control level of agglutination in the absence of Fab was computed. Two replicate wells were used per treatment. Protein concentration was determined by the Lowry method using BSA as a standard.

purified pallidin or crude extracts of *P. pallidum* cells. IgG was purified by ammonium sulphate precipitation followed by DEAE-cellulose chromatography¹¹. Fab fragments were derived by papain digestion and purified by the standard procedure¹². Control Fab fragments were prepared in parallel from normal rabbit serum.

The Fab fractions were assayed for their ability to inhibit haemagglutination produced by purified pallidin in a standard assay. The reaction mixture added to the wells of a Microtiter V plate was as follows: 25 μ l of a twofold dilution series of pallidin in 75 mM NaCl, 75 mM $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ (pH 7.2 PBS), 25 μ l of formalinised human O erythrocytes 2.5% (v/v) in PBS; and 25 μ l of Fab diluted in 16.7 mM $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$, 10 mM EDTA (pH 6.2). At a final concentration of 0.5 $\mu\text{g ml}^{-1}$, immune Fab reduced the haemagglutination titre by 50%: normal Fab did not produce inhibition at concentrations up to 250 $\mu\text{g ml}^{-1}$.

Immune Fab (at 3 $\mu\text{g ml}^{-1}$) directed against pallidin inhibited the cohesiveness of *P. pallidum* cells by 50%, whereas control Fab had little effect (Fig. 2). Inhibition of agglutination was also obvious on microscopic inspection.

Beug and colleagues^{13,14} have demonstrated that Fab fragments from antisera against crude fractions of differentiated *D. discoideum* amoebae block cohesion of *D. discoideum* cells; but in this case the cell-surface antigens with which the contact-blocking Fab fragments react are not known. In the present experiments, Fab fragments directed against the cell-surface lectin, pallidin, were shown to inhibit cohesiveness of differentiated *P. pallidum* cells, as measured in a gyrated suspension. Although further experiments are required to fully define the morphogenetic role of the cellular interactions measured by our cohesiveness assay, we assume that this experimentally defined cohesiveness is functionally significant, since it is correlated with the ability of cells at different developmental steps to form stable intercellular contacts when moving on a solid substratum. Together with other evidence summarised here and reviewed elsewhere¹⁵, the present experiments provide strong support that stage-specific intercellular adhesion is mediated by the cell-surface lectin, pallidin.

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New approach to determination of specific functions of platelet membrane sites

It has been suggested that the layer of bound carbohydrate on the surface of a platelet contains groupings vital to the haemostatic function of the platelet¹. This haemostatic role is largely dependent on the ability of the platelet to adhere to exposed subendothelial components in the event of vessel damage². Platelet aggregation, induced by bovine factor VIII and ristocetin, and platelet adhesion to subendothelium are impaired in the Bernard-Soulier syndrome, while aggregation induced by ADP is apparently normal^{3,4}. A gross abnormality in the 155,000-molecular weight glycoprotein seen in membrane fractions prepared from platelets from this syndrome⁵ suggests that the glycoprotein acts as an acceptor-receptor during the interaction with subendothelium and macromolecular aggregation-inducing agents. Here we report further evidence that a specific site on the platelet is required for ristocetin and bovine factor VIII to induce platelet aggregation and for platelet adhesion when aggregation and platelet adhesion to subendothelium are inhibited by an acquired antiplatelet antibody found in a polytransfused Bernard-Soulier patient. This antibody induced *in vitro*, on normal human platelets, a specific Bernard-Soulier-like defect, so that adhesion to subendothelium was impaired and aggregation with ristocetin and bovine factor VIII was defective, while ADP-mediated aggregation was unaffected.

The antibody, which was shown to be an IgG, was isolated from the plasma of a Bernard-Soulier patient (P) who had received multiple transfusions of platelet concentrates during the arrest of a bleeding episode. It seemed to differ from the usual antiplatelet alloantibodies: a negative complement fixation test was observed with a panel of 25 control human

Fig. 1 Effect of different dilutions of the antibody from patient P on the aggregation of control human PRP induced by ADP, collagen, bovine factor VIII and ristocetin. Citrated control human PRP (0.2 ml) was incubated for 60 s in the cuvette of a Born MK II miniaggregometer (produced by the Department of Pharmacology, Royal College of Surgeons, London) in the presence of several different non-agglutinating dilutions of the antibody (in saline) before addition of 10 μ l of one of the following inducers (final concentration): $\blacktriangle$, 0.6 μmol of ADP (Sigma); $\times$, 20 μg of collagen (Stago, Asnieres); $\square$, 160 μg of bovine factor VIII (AB Kabi, Stockholm); $\circ$, 200 μg of ristocetin (Lundbeck, Copenhagen). Results (mean values) of 10 experiments are expressed as the percentage inhibition of the aggregation intensity in the absence of antibody.

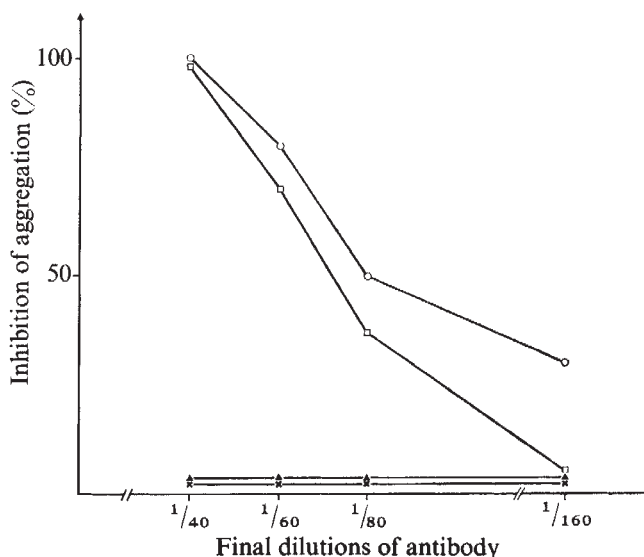


Table 1 Consumption studies of the antibody from patient by platelet stroma

Antibody final dilution	Residual agglutinating activity with control PRP after incubation with				Residual inhibitory effect on ristocetin-induced aggregation of control PRP after incubation with			
	Saline	Control platelet stroma	B S 1 platelet stroma	B S 2 platelet stroma	Saline	Control platelet stroma	B S 1 platelet stroma	B S 2 platelet stroma
1:20	50	12	60	55				
1:40	15	0	18	16	80	10	45	50
1:80					45	5	20	45

Hypercitratized PRP from control human or two other Bernard-Soulier patients, BS1 and BS2, were centrifuged for 10 min at 2,000g and 15 °C. Platelets pellets were resuspended in 0.015 M Tris-HCl buffer, pH 7.4, containing 0.135 M NaCl, 0.053 M KCl and 0.015 M EDTA. These pellets washed twice in this buffer, then finally resuspended in the same buffer without EDTA, were disrupted by five times freeze-thawing and washed twice in saline (27,500g, 15 min, 15 °C) to remove most of the intracellular constituents. The stromas were resuspended at a concentration of 1 mg of protein per ml in saline. Samples (0.2 ml) of this membrane-enriched fraction or 0.2 ml of saline were incubated with 0.2 ml of different dilutions of 6 mg of the antibody solution (final dilutions of the antibody were 1:20, 1:40, 1:80) for 120 min at 37 °C, followed by overnight incubation at 4 °C. After centrifugation at 27,500g for 15 min at 15 °C, the supernatants were tested for their residual agglutinating activity (left hand side) on control PRP (results expressed as percentage of agglutination) and inhibitory effect on ristocetin-induced aggregation (right hand side) of control PRP (results expressed as percentage of inhibition of the intensity of ristocetin aggregation). Mean values of three experiments.

platelets by a microtechnique described before⁶, the lymphocytotoxicity test of Terasaki and McClelland⁷ revealed only a weak anti-HLA-A1 antibody, and the thromboagglutination test⁸ was positive on 20 control human platelets whatever the HLA, PLA₁, KOa or KOb type. When control citrated platelet-rich plasma (PRP) from 50 donors was incubated at 37 °C and stirred in a Born MK II miniaggregometer cuvette in the presence of the antibody, agglutination was observed after about 1 min. The intensity of the agglutination of control PRP was directly related to the concentration of antibody added. No agglutination was observed with a final dilution greater than 1:40. PRP from a patient with severe quantitative von Willebrand disease was normally agglutinated by the antibody. In contrast no agglutination effect (at any dilution) was observed when the antibody was added to PRP obtained from two other Bernard-Soulier patients. Using PRP from the family of one of these patients, we found that the agglutinating effect of the antibody from patient P was nil for an apparently healthy brother, about 60% of normal for another healthy brother and also for the mother and the father, making possible a distinction between the normal and heterozygous state.

Figure 1 shows that the antibody from patient P was a strong inhibitor of aggregation induced by ristocetin and bovine factor VIII. The inhibitory effect was related to the concentration of antibody used. In contrast, ADP or collagen-induced aggregation were not inhibited by the antibody. Table 1 shows that after incubation with control platelet stroma, both agglutinating activity and inhibition of ristocetin-induced aggregation by the antibody were suppressed, while with the platelet stroma of the two other Bernard-Soulier patients,

much of the antibody activity was recovered in the supernatant. In the presence of antibody dilutions that inhibited 100% of control PRP ristocetin-induced platelet aggregation, platelet adhesion to subendothelium was estimated by the Baumgartner technique² to be 24% compared with 72% without antibody (Table 2).

The agglutinating effect of the antibody from patient P on control human PRP and not on the two other Bernard-Soulier PRP, could indicate that the antibody was an antiplatelet antibody directed against an antigen present on all normal platelets but absent, very reduced or abnormal on Bernard-Soulier platelets. This suggestion is supported by the fact that control human platelet stromas consumed the antibody while Bernard-Soulier platelets were much less efficient in doing so. Using subagglutinating dilutions of the antibody from patient P, we have demonstrated the strong and specific inhibition of aggregation induced by ristocetin and bovine factor VIII while ADP and collagen aggregation were not influenced. In the presence of the antibody, adhesion of normal platelets to subendothelium was much reduced; this may indicate that the antigen recognised by the antibody was necessary for the aggregation inducing agents bovine factor VIII and ristocetin, and for adhesion to subendothelium, but not for aggregation mediated by ADP and collagen. The antibody was not directed against von Willebrand factor, for it induced normal agglutination of PRP from severe quantitative von Willebrand disease. Furthermore, von Willebrand factor activity in ristocetin-induced platelet aggregation was normal in the fraction eluted in the void volume after gel filtration on Sepharose 2B of plasma from patient P³.

As the platelets of patient P had a surface defect involving a membrane glycoprotein⁴, we suggest that the antibody is directed against a specific platelet site, implicated in the interaction with subendothelium and macromolecular aggregation-inducing agents. This antibody makes possible a distinction between the surface groupings involved in aggregation induced by ADP and collagen on the one hand, and in aggregation induced by ristocetin and bovine factor VIII and adhesion to subendothelium on the other hand. The platelet antigen recognised by the antibody could be the 155,000 molecular weight glycoprotein, but the exact antigenic site of the molecule remains unknown and is under investigation.

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Table 2 Effect of antibody from patient P on interaction of platelets with rabbit aorta subendothelium

Antibody final dilution	Interaction of platelets with aorta subendothelium	
	Naked	Adhesion
0	28±12	72±12
1:80	43	57
1:40	76	24

The procedure was as described by Baumgartner². Everted segments of rabbit aorta, previously denuded of endothelium were mounted on the central rod of a perfusion chamber. Citrated whole blood from donors was first incubated for 10 min with the antibody (final dilutions 1:40, 1:80) or with saline before being circulated for 10 min at 37 °C with an average flow rate of 160 ml min⁻¹ through the chamber. After the chamber had been rinsed with phosphate buffer, the blood vessel segments were fixed and embedded in Epon for examination by light microscopy and for evaluation of platelet interaction with subendothelium. Results are expressed as the percentage of the subendothelial surface that is devoid of platelets (naked) or that is covered by adherent platelets (adhesion). Results of control without antibody are given as the mean values of 10 experiments ± 1 s.d.

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Kinetics of agonist-induced intrinsic fluorescence changes in membrane-bound acetylcholine receptor

ACETYLCHOLINE receptor (AChR)-rich membranes derived from fish (*Electrophorus* and *Torpedo*) electric organs have been used extensively for *in vitro* binding and functional studies¹. These membrane fragments (microsacs) are particularly suited for physical investigations of AChR–ligand interactions because of their inherently high content of receptor, retained in its natural environment. A number of extrinsic fluorescent probes have been applied to the determination of binding specificity, equilibria and, in a preliminary way, kinetics at the level of the isolated membrane, as well as the neuromuscular junction and purified receptor^{1–6}. We report here the existence of intrinsic protein fluorescence changes, induced in membrane fragments from *Torpedo marmorata* electroplaques by the natural neurotransmitter acetylcholine. We have used highly sensitive fluorescence kinetic techniques to obtain the concentration dependence and some of the corresponding equilibrium and

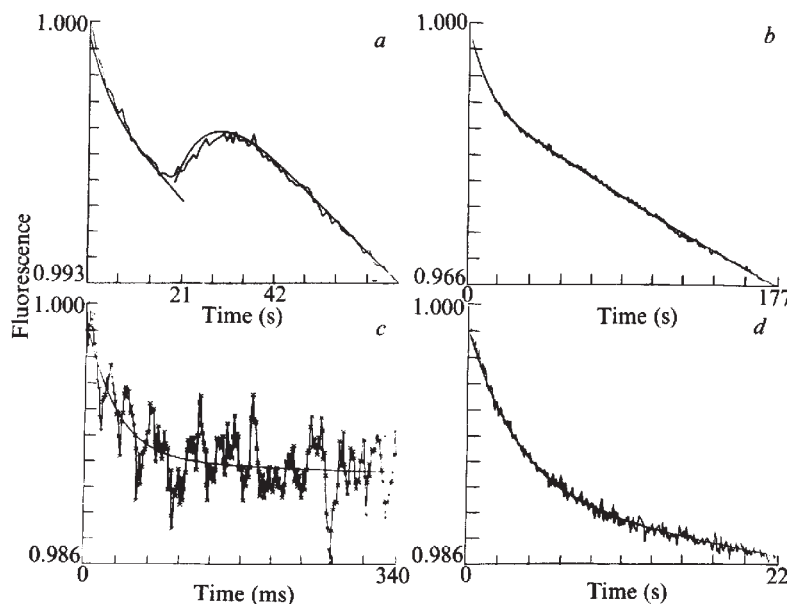
rate constants for the initial binding and subsequent isomerisation reactions. The latter can be correlated with electrophysiological data and the functional properties of the post-synaptic membrane^{7–9}, particularly with respect to the process of desensitisation, and with other quantitative results obtained with the same microsome preparations¹⁰. In particular, our data provide physical evidence for the existence of at least three conformational states of the cholinergic receptor protein *in situ*, interrelated by reversible kinetic processes.

AChR-rich microsacs were prepared essentially as described by Cohen *et al.*¹¹, and their α -toxin-binding capacity determined by the Millipore filter assay¹². Fluorescence kinetic experiments were carried out in the stopped-flow apparatus designed in this laboratory¹³. Further experimental details are given in the legends to Figs 1 and 2.

Figure 1 shows the changes in the intrinsic fluorescence of the membrane-bound AChR induced by mixing with the natural neurotransmitter, ACh, and another cholinergic agonist, carbamylcholine. An exponential decay in fluorescence was observed on the addition of more than 10 μ M ACh to the microsacs (Fig. 1a). This initial response reversed to the original fluorescence decay after a delay which increased with increasing ACh concentrations or with a decreasing acetylcholinesterase (AChE) content of the microsacs. The decay, and its reversal, were superimposed on a uniform linear decrease in fluorescence emission probably due to photolysis caused by the high illumination intensities in the stopped-flow apparatus. (The drift could be determined easily and corresponded to a -0.012% s^{-1} relative change in fluorescence—that is, much slower than the ligand-induced effect. All records were corrected appropriately.)

At ACh concentrations $< 2 \mu$ M, the initial response was no longer monoexponential and its magnitude was markedly reduced, presumably due to rapid degradation of the ligand by the AChE present in the partially purified microsacs. In the presence of small amounts of the AChE inhibitor paraoxon

Fig. 1 Agonist-induced changes in the intrinsic fluorescence of AChR-rich microsacs from *T. marmorata* followed by stopped-flow fluorimetry. The apparatus designed in this laboratory^{13,14} was used on-line to a PDP 11/20 minicomputer for real-time data acquisition. The signal measured after mixing membranes and ligand was the quotient of the fluorescence emitted above 320 nm (Schott WG filter, Mainz) to a part of the exciting light passing through a 0.25-m Jarrell-Ash monochromator and a 295.1-nm interference filter (Corion, Boston). Six lines of 256 points were acquired, each point representing the signal averaged over increasingly longer time intervals (0.2 ms–1 s). The decay curves were analysed with a nonlinear regression programme written by R. Clegg. *a*, Response of partially purified microsacs to the mixing with ACh in the stopped-flow fluorimeter (normalised average of seven records). The specific activity of the membrane fragments was 530 pmol α -toxin sites per mg protein. The esterase activity was much higher than in purified AChR-rich microsacs¹¹—that is, equivalent to 8% of the same protein concentration of purified AChE (E.C. 3.1.1.7) from *T. californica* (a gift from Dr P. Taylor, Univ. California, San Diego). Concentrations after mixing: 69 μ M ACh, 20 nM receptor sites. (An excess of ligand over AChR was used in all experiments, thus satisfying pseudo-first-order conditions.) *b*, Normalised average of five stopped-flow records of ACh-induced changes in the presence of 150 nM paraoxon. The record includes linear baseline drift. Specific activity was 1,000 pmol per mg protein. Concentrations after mixing: 4.1 μ M ACh, 20 nM AChR sites. The smooth curve corresponds to an exponential decay with $\tau_{11} = 9.7$ s and $\alpha_{11} = -1.02\%$. No reversal was observed in the absence of AChE activity.



c, Normalised record of seven stopped-flow experiments of response to ACh in the presence of 150 nM paraoxon. Concentration after mixing: 12.3 μ M ACh and 20 nM AChR sites. The theoretical curve corresponds in this case to an exponential decay with $\tau_1 = 33$ ms and $\alpha_1 = -0.77\%$ (note time scale) after subtraction of the slower exponential component with $\tau_{11} = 7.9$ s and $\alpha_{11} = -1.04\%$

(not shown). *d*, Response to carbamylcholine. Normalised average of seven stopped-flow records. Concentrations after mixing: 180 μ M carbamylcholine and 20 nM AChR sites. The smooth curve corresponds to a fitted exponential with $\tau_{11} = 3.6$ s and $\alpha_{11} = -0.93\%$ (the baseline linear drift was determined from the continuation of the record to 177 s).

(diethyl-*p*-nitrophenylphosphate, 150 nM), however, the ACh-induced fluorescence quenching could be followed over a wide concentration range (Figs 1b and 2).

The relaxation times and amplitudes characterising the progress curves after mixing were concentration dependent but reached plateau values at high ACh levels (Fig. 2). Over a limited concentration range, an additional faster exponential decay in fluorescence was seen (Figs 1c and 2) but was not detectable elsewhere, for reasons given below.

Similar kinetic phenomena, except for the reversal were observed with another agonist, carbamylcholine, which is not hydrolysed by the esterase (Fig. 1d). The relaxation time and amplitude, however, reached constant values at significantly higher concentrations of carbamylcholine as compared with ACh (65 against 4 μ M, respectively).

The specificity of the ligand-induced fluorescence changes and their attribution to distinct states of the receptor protein follow from findings that: the effects were abolished by pre-incubation with a tenfold molar excess of α -cobrotoxin; and the total amplitude of the fluorescence quenching was proportional to the specific activity (in terms of toxin binding) of various microsac preparations and not to the total protein or AChE content. The essential reversibility of the phenomenon—that is, the return of the AChR to its initial resting state—is attested to by the reversal observed as a consequence of dissociation and enzymatic hydrolysis of the free transmitter. The onset time and time course of the reversal were in good agreement with expectation on the basis of AChE determinations using acetylthiocholine¹⁴.

The simplest reaction mechanism consistent with our experimental results consists of a fast binding step followed by a slow isomerisation



where A is the agonist and R and D are two distinct conformers of the AChR. The experimentally observed slow relaxation time, τ_{II} , corresponds in this sequential scheme to the equilibration of the second step to which the first is thermodynamically coupled

$$(\tau_{II})^{-1} = \left[\frac{A}{K_1 + A} \right] k_3 + k_4 \quad (2)$$

where K_1 is the dissociation constant (k_2/k_1). The reciprocal relaxation (Fig. 2) has an ordinate intercept of k_4 , an initial slope of k_3/K_1 and a limiting value at high [A] of $k_3 + k_4$. The corresponding amplitude (in terms of relative fluorescence) is given by

$$\alpha_{II} = \alpha_{total} - \alpha_I = \left[\frac{A}{K_1 + A(1 + K_2)} \right] (\Delta\Phi_1 + K_2\Delta\Phi_2) - \left[\frac{A}{K_1 + A} \right] \Delta\Phi_1 \quad (3)$$

where $\Delta\Phi_1$ and $\Delta\Phi_2$ are the fractional changes in fluorescence on passing from state R to states AR and AD, respectively, and K_2 is the isomerisation equilibrium constant (k_3/k_4). α_{II} has an ordinate intercept of zero, an initial slope of $K_2\Delta\Phi_2/\Delta\Phi_1$, a maximum magnitude at $[A] \sim K_1(K_2\Delta\Phi_1/\Delta\Phi_2)^{-1}$ and a plateau value of $K_2(\Delta\Phi_2 - \Delta\Phi_1)/(1 + K_2)$.

Using the above formulation, it was possible to fit the data obtained at various concentrations of ACh or carbamylcholine with one set of parameters for each agonist (Fig. 2 and Table 1). It is seen that carbamylcholine has a lower affinity for the R state of the receptor but the transition from the AR to the AD state is more rapid and extensive (k_3 and K_2 larger than for

ACh). The fluorescence parameters, however, are about the same for both drugs, and indicate that the emission of the receptor in the states R, AR, and AD decreases in that order. The values of K_1 for both ACh and carbamylcholine agree well with the apparent dissociation constants derived from [²²Na] efflux measurements in the microsac preparation and from direct binding to solubilised receptor¹⁰.

The limited data for the rapid decay process (Figs 1 and 2) do not suffice for the determination of the corresponding rate constants, although k_1 is certainly $> 10^6 \text{ M}^{-1} \text{ s}^{-1}$. Thus, at low ACh concentration (for example, 1 μ M), α_I is too small ($\leq 0.2\%$), whereas at high concentrations ($> 40 \mu$ M), τ_I is too small ($< 10 \text{ ms}$) for the relaxation to be perceptible directly. Its presence is indicated nevertheless by the decrease in α_{II} at high levels of ACh (Fig. 2).

The addition of a further rapid isomerisation step between an inactive complex AR (equation 1) and a conducting form AR' does not alter the ability of the above formulation to account for the data of Fig. 2 (except to change the quantitative interpretation of K_1 and k_3). Such a scheme is required to rationalise the submillisecond rise time of the endplate potential^{8,15} and the analyses made of potential fluctuations (membrane noise)^{16,17}. If the AR-AR' equilibrium is rapidly established and particularly if it favours the AR state¹⁷, we would fail to detect it due to the 3–5 ms time resolution of the conventional stopped-flow apparatus³¹, even if a distinct fluorescence change were involved.

The second relaxation process is most likely related to the formation of an inactive or 'desensitised' state of the receptor (AD), a phenomenon which occurs in *T. marmorata* *in vivo*¹⁸ and *in vitro*^{10,19,20} and, moreover, generally in the cholinergic synapse^{8,9,21–24}. The rate constant k_3 (Table 1) indicates that desensitisation occurs in the time range of seconds, a finding compatible with most electrophysiological data. In addition, other studies (unpublished results) have shown the expected influence of temperature and of effectors known to influence desensitisation on the fluorescence relaxation process. Although the simple scheme outlined above accounts well for the experimental results, the rapid reversal of the fluorescence decay on ACh hydrolysis (Fig. 1a) suggests the existence of an alternative route for recovery from the desensitised state with a rate $> 0.1 \text{ s}^{-1}$ (and thus incompatible with the value of k_4 , Table 1). This phenomenon, which may to a limited degree represent the physiological situation, is consistent with a cyclic scheme

Fig. 2 Dependence on ACh concentration of the fluorescence decay rates (τ^{-1}) and amplitudes (α) after mixing AChR-rich microsacs with ACh in the stopped-flow fluorimeter. Specific activity: 1,000 pmol α -toxin-binding sites per mg protein. AChR concentration after mixing was 20 nM in α -toxin sites. ■, Decay rate measured in the presence of the AChE inhibitor paraoxon (300 nM); □, decay rate measured in the absence of the AChE inhibitor; ●, observed amplitude of decay in fluorescence in the presence of 300 nM paraoxon; ○, amplitude measured in the absence of paraoxon; ▲, amplitude of fast decay in fluorescence observed at 4 μ M ($\tau_I = 120 \text{ ms}$) and at 12 μ M ($\tau_I = 32 \text{ ms}$) ACh, with the same membrane preparation used in experiment shown in Fig. 1c. Solid lines correspond to the theoretical curves calculated for the sequential scheme (equations (1) and (2)) using the values given in Table 1.

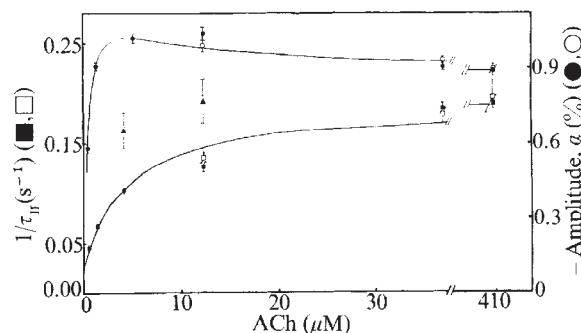


Table 1 Parameters derived from the ligand-induced fluorescence changes using the sequential scheme outlined in equations 1–3

	ACh*	Carbamylcholine
K_1	4.1 μM	66 μM
	2.0 $\mu\text{M}^\dagger$	50 $\mu\text{M}^\dagger$
k_3	0.16 s^{-1}	0.37 s^{-1}
k_4	0.027 s^{-1}	0.01 s^{-1}
K_2	6	37
$\Delta\Phi_1$	-0.67% ‡	-0.75%
$\Delta\Phi_2$	-1.7% ‡	-1.5%

*In the presence of 150 nM AChE inhibitor, paraoxon. Temperature: 20 °C.

† From Table 2 in ref. 10.

‡ This and related values have been normalised to a specific activity of 1 nmol ^3H - α -toxin per mg protein. The measured fractional fluorescence changes, $\Delta\Phi_1$ and $\Delta\Phi_2$, are related to the actual relative changes in the fluorescence of the AChR by a proportionality factor β which denotes the fractional contribution made by the receptor to the total protein emission. That is, the true changes associated with the intrinsic AChR fluorescence are given by $\beta\Delta\Phi_1$ and $\beta\Delta\Phi_2$, where β could have a value ≥ 10 .

extending equation (1) to include the dissociation of the AD complex and the isomeric transition of the D to the R state. Such a mechanism, which was considered originally by Katz and Thesleff²¹ and developed further by Rang and Ritter^{22,23} best fits available pharmacological data^{8,9}. Our results indicate that the desensitised receptor has a much higher affinity for the agonist than does the resting state ($K_3 < K_1/K_2 \ll K_1$, where K_3 , the dissociation constant for the AD state, must be $< 0.3 \mu\text{M}$ to account for the consistency of the data with the sequential scheme).

It is important to note that regardless of the true binding order associated with activation (that is, number of ligand molecules required per functional receptor, a value which may well exceed one^{7–9,25–27}) the isomerisation step (desensitisation) we have observed seems to proceed from a monoliganded state (we cannot exclude the presence of other more tightly bound ligand molecules). This finding suggests that if binding of additional molecules is required for activation, it involves only a small proportion of the available receptors.

Conformational changes in the cholinergic receptor have been invoked to explain drug antagonism (the 'metaphilic effect'²⁸), agonist efficacy^{29,23,7}, agonist-dependent mean channel conductance³⁰, and numerous *in vitro* observations, including those derived using fluorescence probes^{1,3,6}. The advantages of using intrinsic protein fluorescence in such studies are twofold: there is a minimal perturbation of the system; and natural ligands can be used at high concentration without loss of specificity or sensitivity. The magnitudes of the effects we have observed, though small, seem to be specific. Studies now in progress are aimed at characterising the structural changes responsible for the fluorescence states of the AChR.

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Virtual absence of L-glutamate from the haemoplasm of arthropod blood

DURING the past decade substantial evidence has accumulated that L-glutamate is a transmitter at arthropod somatic neuromuscular junctions¹. One of the major paradoxes in the acceptance of this evidence is the reported high concentration of L-glutamate in the haemolymph, which is sufficient on neurophysiological preparations to decrease or abolish neurally-evoked contractions^{2,3}. To overcome this problem it has been suggested that either there is a barrier to L-glutamate between the neuromuscular junction and the haemolymph^{4,5}, or L-glutamate is bound in the haemoplasm in a pharmacologically inactive form⁶ or sequestered into the haemocytes^{6,7}. These suggestions would now seem to be obsolete in view of our evidence that L-glutamate is virtually absent from arthropod haemoplasm.

The neuromuscular junctions of *Lucilia sericata*, in common with other blowfly larvae, are not covered by glial cells^{8,9} and so there is no cellular barrier between the synapse and the L-glutamate in the haemolymph. Injection of L-glutamate into the haemolymph of larval *L. sericata*, as found with larval *Calliphora vicina*⁶, caused reversible paralysis, confirming that there is no apparent barrier to L-glutamate between the haemolymph and the neuromuscular junction in this *in vivo* preparation. Furthermore, injection of radiolabelled L-glutamate into the haemolymph reveals rapid metabolism and removal of L-glutamate to other tissues, which indicates that the L-glutamate is not bound in the haemoplasm in a pharmacologically inactive form. As fresh haemolymph has been reported to be pharmacologically inactive⁵, an investigation into the electrophysiological effects of fresh haemolymph on body wall muscles 6A and 7A of *L. sericata*¹⁰ was undertaken. These muscles are sensitive to L-glutamate which causes muscle membrane depolarisation and contraction. The effect on the membrane potential when fresh haemolymph was applied was unpredictable individually, but when repeated many times gave no significant difference between the membrane potentials before and after haemolymph application ($n = 105$). Aged haemolymph gains pharmacological activity in *Schistocerca gregaria*⁵ and this is also true for aged *L. sericata* haemolymph, which evokes physiological responses similar to those elicited by L-glutamate.

Fresh haemolymph is thus pharmacologically inactive, and no barriers (physical or chemical) seem to exist between the neuromuscular junction and the haemolymph. One possible conclusion is that the haemoplasm does not contain L-glutamate and so to test this hypothesis, we analysed the haemolymph of various insects using a Locarte automatic amino acid analyser (Table 1 and Fig. 1). The haemolymph from individuals of each species was pooled, to give samples of 30 μl which were centrifuged for 4 min at 800g, the haemoplasm removed and the proteins precipitated using mainly 70% ethanol, and occasionally

Table 1 The concentration of L-glutamate and L-glutamine in the haemoplasm and haemocytes of five insects and one crustacean

	<i>Lucilia sericata</i>		<i>Calliphora erythrocephala</i>		<i>Schistocerca gregaria</i>		<i>Locusta migratoria</i>		<i>Periplaneta americana</i>		<i>Carcinus maenas</i>	
Place of extraction	Below spiracular sclerite				Dorsal cervical membrane				Removing hind leg		Membrane between coxa and thorax	
Animals per sample	5		3		3		3		3		1	
No. of samples	C(5)	P(6)	C(4)	P(4)	C(4)	P(8)	C(4)	P(5)	C(6)	P(7)	C(2)	P(5)
Mean L-glutamate concentration	3.3 ±1.5	— 6 _t	20.1 ±7.4	—	0.8 ±0.8* 2 _t	— 1 _t	0.4 ±0.4* 3 _t	— 1 _t	6.3 ±2.6	— 6 _t	9.6 ±2.6	— 3 _t
Mean L-glutamine concentration	121.3 ±50.3	1047.3 ±130.3	131.6 ±29.8	827.0 ±223.0	25.7 ±11.9	403.0 ±22.3	9.2 ±2.8	305 ±41.6	60.6 ±15.6	214.6 ±26.6	15 ±7.6	48 ±15.3

C, Haemocytes; P, haemoplasm. Units, 10^{-5} M ± s.e.

*One sample contained quantifiable L-glutamate.

t, Number of samples containing trace of L-glutamate ($< 10^{-5}$ M).

3% sulphosalicylic acid (glutamate recovery 97–99% and 95–98% respectively). The precipitate was removed by centrifugation, resuspended in distilled water, recentrifuged and the supernatants pooled. All experimental procedures were carried out at 4 °C. The results revealed that although there is L-glutamate in the haemocytes of all the species tested, it is not detectable, or only present in trace amounts ($< 10^{-5}$ M) in the haemoplasm. These results have been confirmed qualitatively by high voltage paper electrophoresis (HVPE), and a Beckman 120C amino acid analyser with an initial buffer of pH 3.25, which achieves a discrete glutamate peak away from the glutamine peak. Our data thus clearly contradict the reports of others^{2–7,12–14} who found levels of L-glutamate as high as

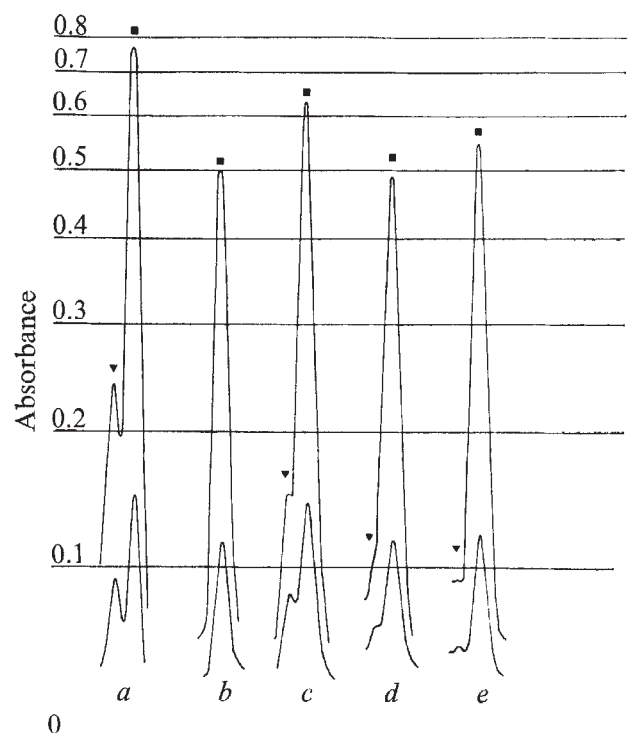
5.8×10^{-4} M (ref. 5) in arthropod haemoplasm. To resolve this contradiction, we studied the methods used by these other workers, and found that these methods are capricious, for the following reasons.

(1) L-glutamine is often the most abundant amino acid in the haemoplasm (10^{-2} – 10^{-3} M) and is known to be unstable^{15–18} at extremes of pH. Using HVPE and radiolabelled L-glutamine, we found that the common acid protein precipitants (perchloric acid, trichloroacetic acid and sulphosalicylic acid) at the quoted concentrations are sufficient to convert some L-glutamine to L-glutamate and pyrrolidone carboxylic acid (Table 2). Furthermore, perchloric acid left at room temperature in a clear glass vessel shows a dramatic increase in hydrolysing ability (Table 2).

(2) Many published haemolymph extractions were conducted at room temperature^{3,5,13}. We found it difficult to prevent the first stages of clotting at room temperature and even at 4 °C half the samples of some species had to be discarded after centrifuging because some clotting had occurred. Haemolymph analysis of these partially clotted samples revealed L-glutamate in the haemoplasm at concentrations as high as 4.5×10^{-4} M. Thus attempts to analyse partially clotted samples of haemolymph of those insects tested will reveal L-glutamate in the haemoplasm. This could explain why aged haemolymph gains pharmacological activity.

(3) Many authors did not separate haemoplasm from haemocytes^{11,19,20}. Too low a centrifugation speed, however, will contaminate the haemoplasm with L-glutamate from the haemocytes. Too high a centrifugation rate on the other hand,

Fig. 1 Traces taken from Locarte automatic amino acid analyser. Upper trace, Absorbance at 570 nm; lower trace, absorbance at 440 nm. *a*, Standards: glutamine, 53 nmol; glutamate, 20 nmol. *b*, Uncollected *Periplaneta americana* haemoplasm showing no detectable glutamate. *c*, Clotted *P. americana* haemoplasm showing presence of glutamate. *d* and *e*, Uncollected *P. americana* haemoplasm showing traces of glutamate ($< 10^{-5}$ M). ■, Glutamine; ▼, glutamate. All peak heights integrated by computer.

**Table 2** Reaction products following treatment of glutamine with various protein precipitants

Treatment	Glutamate	Pyrrolidone carboxylic acid
70% Ethanol	0.3–0.8	1.2–1.7
3% Sulphosalicylic acid	2.0–4.3	2.13–5.93
15% Trichloroacetic acid	0.2–2.2	0.83–5.43
0.4 N Perchloric acid	0.7–1.0	0.83–4.62
12-h-old 0.4 N Perchloric acid	10.1–11.0	10.93–11.73
1-week-old 0.4 N Perchloric acid	14.0–18.0	13.23–17.63

Glutamate and pyrrolidone carboxylic acid are expressed as percentage produced from hydrolysis of 10^{-3} M glutamine. Incubation of glutamine with protein precipitants was for 10 min in an ice bath. The 'aged' perchloric acid had been kept at room temperature. Range given from five separate determinations. The percentages of glutamate and pyrrolidone carboxylic acid formed vary greatly and the above figures should not be taken as absolute but merely as an indication that the reaction occurs.

causes haemocyte rupture, with consequent release of L-glutamate into the haemoplasm.

(4) Other tissues have a high concentration of L-glutamate¹⁴ which may contaminate the haemoplasm during extraction.

Thus our results show that L-glutamate is virtually absent from arthropod haemoplasm, or if detected, exists in concentrations which are far below those measured by other workers. We believe that this new finding resolves one of the major obstacles to the acceptance of L-glutamate as a neurotransmitter at arthropod somatic nerve-muscle junctions. Further details of this work will be published elsewhere.

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footshock either alone or in pairs received, within a Plexiglas enclosure, 50 2-mA footshocks of 0.4 s duration with a fixed intershock interval of 7.5 s. This procedure and apparatus have been described in detail elsewhere^{4,5}. Rats did not successfully avoid the footshock and all rats, whether alone or paired, subjectively appeared to sustain comparable levels of shock as indicated by vigorous vocal and skeletal motor responses. All shocked groups were tested for 3 consecutive days in the morning.

Groups receiving drugs were injected approximately 30-60 min before behavioural testing, or at the end of the study, before killing. The saline group received 0.9% saline, intraperitoneally in a 2-ml volume, for 3 d. The caffeine groups received caffeine given as the citrate in a dose of 100 mg kg⁻¹, in a 2-ml volume, also for 3 d.

Immediately following the third day of testing and treatment, each rat was killed by microwave irradiation, in order to rapidly denature the enzymes responsible for the synthesis (adenylate cyclase) and degradation (phosphodiesterase) of cyclic AMP. The microwave apparatus used was a model 4104 Metabostat (Gerling Moore), with a power output of 5 kW at a frequency of 2,450 MHz. Animals which were shocked, either alone or paired, (with 50 footshocks during a 6.2-min period), were killed between 2-5 min after the cessation of footshock. For irradiation, rats were placed in a cylindrical animal holder. This holder allowed the rats to assume a relatively natural position and placed only the head in the microwave field. There were no difficulties with animal excitation. Irradiation for 1.5 s produced rapid and uniform heating of all brain areas. All control and caffeine-injected rats were killed in the same manner. After irradiation, brains were quickly removed from the skull, frozen on dry ice, and stored at -70 °C until analysed. Tissues were homogenised in 5% TCA, containing a known amount of tritiated cyclic AMP to monitor nucleotide recovery during purification. Cyclic AMP was assayed according to a modification of the competitive protein binding method as described by Tovey *et al.*⁶ using a commercially available cyclic AMP-dependent protein kinase (Sigma).

Whole brain levels of cyclic AMP are presented in Table 1. The untreated-unshocked control group (C) had brain levels of 0.202±0.011 (pmol cyclic AMP per mg of wet brain weight). The saline-injected unshocked group (S) had comparable levels of 0.206±0.019. Either shock to rats alone (Escape-attempt) or the administration of caffeine to unshocked rats (CAF) elicited respective cyclic AMP levels of 0.243±0.013 and 0.242±0.027. Paired shocking of rats, eliciting shock-induced aggression, produced levels of cyclic AMP in the untreated (Fighting) and caffeine-treated (CAF-Fighting) groups of 0.445±0.073 and 0.401±0.021, respectively. A one-way analysis of variance for these data was significant at the *P* < 0.005 level (*F*_{5,39} = 8.68). Untreated shock-alone group (Escape-attempt) and the untreated shock-paired group (Fighting) significantly differed from the untreated-unshocked control group by two-tailed *t* test (*P* < 0.05 and *P* < 0.01 respectively). The caffeine-treated group, unshocked, did not significantly differ from the saline-injected group in this study. The shock-paired caffeine-treated group (Caffeine-Fighting) had significantly

Influence of social setting on the induction of brain cyclic AMP in response to electric shock in the rat

RATS subjected to electric footshock display two disparate behaviours, Escape-attempts or aggressive attack, depending on whether the rat is shocked alone or with a conspecific¹. Different responses in blood pressure¹, ACTH levels², and brainstem noradrenaline turnover³ develop in these two patterns of behaviour. On the basis of these studies we hypothesised an additional differential response for brain cyclic AMP levels. We now report a 100% increase in whole brain cyclic AMP following shock-induced fighting but not after identical footshock given to isolated rats. This increase occurred with or without systemic pretreatment of the rats with caffeine, an inhibitor of phosphodiesterase, the enzyme which deactivates cyclic AMP.

Forty-eight male Sprague-Dawley rats (180-220 g, Simonsen, Gilroy, California) were individually housed with *ad libitum* access to food and water and then randomly assigned to one of six experimental groups. These were a control group; a saline-injected control group; a shock-alone (Escape-attempt) group; a shock-paired (Fighting) group; a caffeine-treated group, unshocked; and a caffeine-treated group which was shocked when paired (Caffeine-Fighting). After two weeks of habituation to their environment the rats were exposed to testing. Those rats which received

Table 1 Levels of brain cyclic AMP (pmol per mg wet weight)

Group	N	cyclic AMP (mean ± s.e.m.)
C	7	0.202±0.011
S	7	0.206±0.019
CAF	7	0.242±0.027
Escape-attempt	8	0.243±0.013
Fighting	8	0.445±0.073
CAF-Fighting	8	0.401±0.021

elevated cyclic AMP when compared to the saline-injected ($P < 0.001$) or caffeine-injected ($P < 0.05$) unfought groups. There was no statistically significant difference between the uninjected or saline-injected control groups, or between the caffeine-treated or non-treated fighting groups. The uninjected Fighting group had markedly higher levels of cyclic AMP than the uninjected Escape-attempt group ($P < 0.02$).

These results are in accord with other studies which have demonstrated physiological, neurohumoral, and neurochemical differences elicited by footshock when delivered to rats alone, inducing primarily attempted escape behaviour (a fear response) and when delivered in the presence of a conspecific, inducing fighting behaviour (an aggressive response). Williams and Eichelman demonstrated that the tail blood pressure of the rat decreased in the case of the Fighting paradigm and increased in the case of the Escape-attempt paradigm¹. They also showed that the decrease could be selectively abolished by central destruction⁷ or peripheral destruction (unpublished observations) of catecholamine neurones with 6-hydroxydopamine. Conversely the increase of tail blood pressure in the Escape paradigm could be abolished with adrenalectomy (Williams and Eichelman, unpublished observations). Conner *et al.*² demonstrated that the Escape-attempt paradigm elicited greater ACTH secretion than the Fighting paradigm, consistent with the adrenal-blood pressure response noted by Williams and Eichelman. Stolk *et al.*³ have reported that brainstem noradrenaline metabolism also differs in the two paradigms. Rats shocked on their own (Escape-attempt) demonstrate an increase in noradrenaline turnover in the medulla-pons during the shock period, whereas rats paired and fighting fail to demonstrate this and show an increase in noradrenaline turnover in the 1-h period after shock, which correlates with the number of attacks observed.

The enhancement of shock-induced fighting in the rat has been repeatedly associated with a facilitation of central catecholaminergic systems^{8,9}. Studies in mice have shown interstrain differences in brain cyclic AMP which are genetically controlled and correlate closely with murine aggressive behaviour¹⁰. Additional evidence has accumulated to link cyclic AMP with central catecholamine metabolism and behaviour¹¹. The large increase in cyclic AMP with fighting is consistent with the multiple associations of aggression and cyclic AMP with central catecholamine metabolism. A potential causal and temporal relationship between the increase in cyclic AMP and increased noradrenaline turnover following fighting could be explored in future studies in the light of the increase in noradrenaline turnover following fighting observed by Stolk *et al.*³.

The increase in cyclic AMP following footshock in the Escape paradigm should not be overlooked. It remains dwarfed, however, when compared with the magnitude of increase in the Fighting situation. Although our data in this study fail to demonstrate an increase in whole brain cyclic AMP after three daily injections of caffeine, it must be considered that an increase might have been statistically significant had a larger sample been used or a different time course for sacrifice chosen. Behavioural work¹² has shown that the greatest facilitation of shock-induced fighting following caffeine treatment occurs 4 h after injection. Caffeine given under the conditions of our study neither suppressed nor facilitated the induction of whole brain cyclic AMP; rather, the behavioural setting and the behaviour arising out of it appeared prepotent. Our findings may have further implications for understanding mammalian coping and aggressive behaviour.

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Spike after-hyperpolarisation of a sympathetic neurone is calcium sensitive and is potentiated by theophylline

IN many neurones, calcium as well as sodium enters during the action potential¹. Intracellular injection of calcium has been shown in several neurones to increase membrane conductance to potassium^{2,3}, and voltage clamp experiments have demonstrated that inward calcium currents can activate outward potassium currents^{4–7}. The action potential in many nerve cells is followed by a slower after-hyperpolarisation (AH), which results from increases of one or more potassium conductances^{8,9}. Therefore, calcium influx during an action potential might activate part or all of the subsequent AH^{2,3,10}. A calcium-dependent component of the AH has been demonstrated so far in myenteric plexus neurones^{11,12} and spinal motoneurones¹³. This component of the AH is blocked by Co^{2+} , Mn^{2+} and La^{3+} , which antagonise calcium influx^{12,13}, but agents that potentiate such AHs have not been reported. We have used sucrose-gap recording¹⁴ to investigate the sympathetic ganglion of the bullfrog, and report here that the sympathetic neurones have a calcium-sensitive component of the spike AH. Furthermore, this calcium-sensitive potassium conductance is potentiated by theophylline, a drug known to affect cellular calcium metabolism.

Figure 1 shows that 5 mM theophylline considerably and reversibly potentiated the action potential AH in the sympathetic ganglion. The AH was increased whether the action potentials were generated by orthodromic B (Fig. 1a) or C fibre stimulation or by antidromic stimulation of the cells (Fig. 1b). The predominant effect of theophylline was an enhancement of the AH duration. It increased the half-time for decay of the antidromically elicited AH up to 800%. The duration of the potentiated AH was inversely dependent on stimulus frequency, with maximal durations observed only when stimuli were administered more than 1 min apart. Theophylline enhanced the AH amplitude to a lesser extent, only increasing the peak amplitude of the antidromically elicited AH up to 150%. Additionally, theophylline often changed the AH shape from a smooth curve to one with an inflection point on the decaying slope (Fig. 2b) or, at times, to a biphasic W-shaped curve with early transient and late slow components. The AH reached maximum potentiation within 15 min of the introduction of the drug and

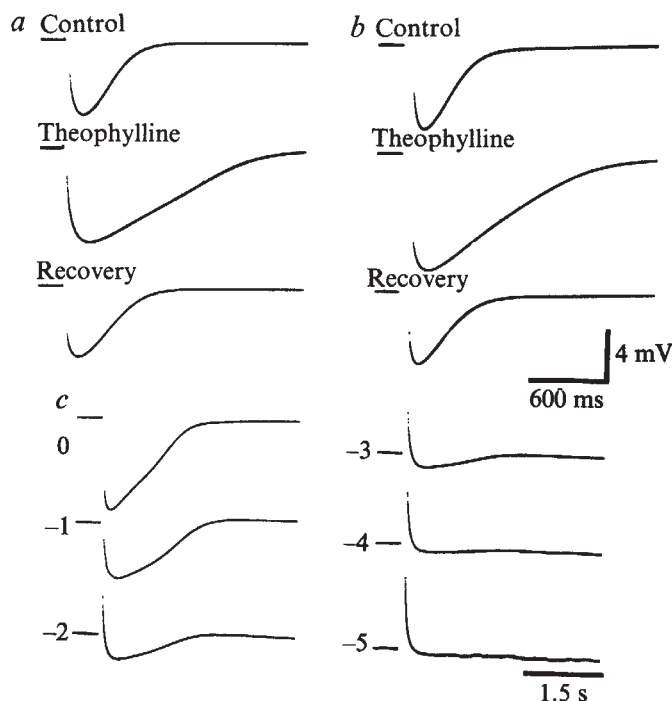


Fig. 1 Effect of theophylline on the action potential AH. *a*, Upper: AH of synaptically activated action potential. Pre-ganglionic B fibres stimulated in the sympathetic chain rostral to the seventh ganglion with a single supramaximal stimulus. Middle: effect of theophylline on the AH 35 min after start of superfusion with Ringer solution containing 5 mM theophylline. Lower: AH 65 min after start of theophylline washout. *b*, Upper: AH of antidromically activated action potential. Postganglionic axons stimulated across the sucrose gap with a single stimulus. Site of stimulation at point where postganglionic axons exit ganglion and enter sucrose chamber. This is termed direct stimulation by Nishi and Koketsu¹⁴. Middle: effect of theophylline on the AH 30 min after start of superfusion with Ringer solution containing 5 mM theophylline. Lower: AH 60 min after start of theophylline washout. *c*, Electrical polarisation of theophylline-potentiated AH. Action potential elicited as in *a* and recorded 50 min after start of superfusion with Ringer solution containing 5 mM theophylline. Hyperpolarising current passed across sucrose gap¹⁴. Numbers to the left of each record indicate the hyperpolarising current $\times 10^{-6}$ A. Records in *a*, *b*, and *c* are from photographs of oscilloscope traces. Action potentials were lost in photographic reproduction. The experiments reported here were conducted on the tenth paravertebral ganglion of the bullfrog (*Rana catesbeiana*) by means of sucrose-gap recording¹⁴. The composition of the Ringer solution was (mM): NaCl, 100; KCl, 2; CaCl_2 , 1.8; Tris-HCl, 16, pH 7.2; and glucose, 1 g l^{-1} . Calcium-free Ringer had the same composition except that CaCl_2 was omitted. The ganglion was continuously superfused with oxygenated Ringer solution.

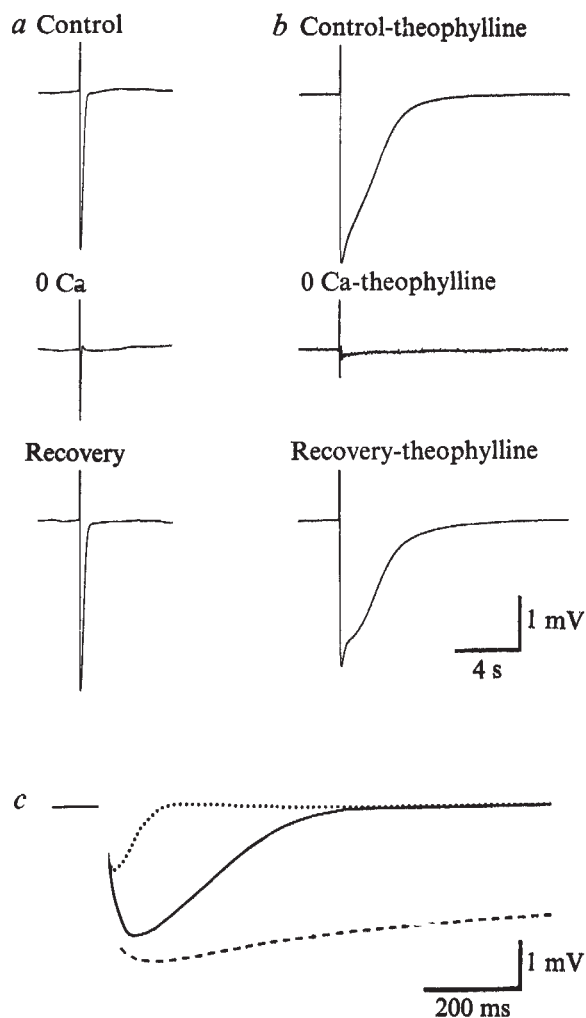
returned to control after superfusion with normal Ringer for 15–30 min. Theophylline did not significantly affect the amplitude of the action potential elicited by orthodromic stimulation. Stimulus artefact obscured the antidromically elicited action potentials.

Theophylline also potentiates the slow inhibitory post-synaptic potential (slow i.p.s.p.) in both bullfrog (our work, in preparation) and rabbit¹⁵ sympathetic ganglia. It is therefore important to compare these two hyperpolarising potentials. The slow i.p.s.p. in bullfrog ganglia is generated by a decrease in sodium conductance and thus is potentiated by moderate hyperpolarising current¹⁶. On the other hand, the action potential AH in frog ganglion cells has been shown to be generated by an increase in potassium conductance, and thus is decreased by hyperpolarising current^{17,18}. Figure 1c shows that moderate hyperpolarisation of the resting membrane potential progressively reduced and abolished the theophylline-potentiated AH. Amplifier limitations prevented the passing of further hyperpolarising current. Furthermore, although atropine ($1 \mu\text{g ml}^{-1}$) blocks the slow

i.p.s.p.¹⁹, it does not affect the theophylline-potentiated AH. These data distinguish the theophylline-potentiated AH from the slow IPSP and suggest that the potassium conductance during the AH is prolonged by theophylline.

The theophylline-induced potentiation of the AH was abolished by the removal of extracellular calcium. This was shown in two ways. First, as illustrated in Fig. 2b, the ganglia were superfused with a Ringer solution containing normal calcium and 5 mM theophylline until maximal AH potentiation occurred. Then they were superfused with Ringer containing theophylline but no calcium. As can be seen, the AH was markedly diminished in duration and amplitude, becoming even smaller than the pre-theophylline control. The potentiated AH returned when calcium was

Fig. 2 Effect of calcium-free Ringer solution on normal and theophylline-potentiated AH. *a*, Normal AH. Upper: AH of antidromic action potential; Middle: effect of calcium-free (0 Ca) Ringer 43 min after start of superfusion with calcium-free Ringer solution; Lower: recovery record 10 min after return to Ringer solution containing normal calcium. *b*, Effect of calcium-free Ringer on theophylline-potentiated AH. Upper: AH of antidromic spike potentiated by 5 mM theophylline; Middle: effect of calcium-free (0 Ca) Ringer containing 5 mM theophylline 33 min after start of superfusion with calcium-free Ringer solution; Lower: recovery record, 11 min after return to Ringer solution containing normal calcium and 5 mM theophylline. Records in *a* and *b* from rectilinear pen recorder (Brush Model 280). Transient depolarisation consists of stimulus artefact and action potential; it was truncated for purposes of illustration. *c*, Effect of theophylline and calcium-free Ringer on AH on expanded time scale. —, Normal AH of antidromic spike; , effect of calcium-free Ringer on normal AH; — — — —, effect of 5 mM theophylline on normal AH. Tracings of oscilloscope records. From the same experiment as *a* and *b*.



restored. Second, ganglia were superfused with calcium-free Ringer for more than 4 h. At that time the addition of 5 mM theophylline did not significantly alter the antidromically elicited AH.

Figure 2a shows that the AH elicited by antidromic stimulation in normal Ringer decreased in duration and amplitude during superfusion with calcium-free solution. This effect was reversed on readdition of calcium to the superfusate. The AH elicited in calcium-free Ringer resembled that elicited in 5 mM theophylline-calcium-free Ringer (Fig. 2b) although there was some variability in the comparative amplitudes from experiment to experiment.

Figure 2c compares the AH, on an expanded time scale, in calcium-free Ringer, in normal calcium Ringer, and in normal calcium with 5 mM theophylline, as recorded by the sucrose gap¹⁴. This technique records the membrane potentials of the population of ganglion cells. In experiments with intracellular recording from sympathetic ganglion cells, the removal of extracellular calcium markedly decreased the duration but not the amplitude of the AH (our unpublished observations with J. Schulman). This suggests that the decrease in AH amplitude in calcium-free Ringer recorded by the sucrose-gap technique is due to the summation of a temporally dispersed population response.

Our data show that the repolarisation phase of the AH in frog sympathetic ganglion cells depends on extracellular calcium. Koketsu and Nishi²⁰ demonstrated previously that calcium as well as sodium can enter these neurones during an action potential. This suggests that calcium influx during the action potential activates a potassium conductance that prolongs the duration of the AH. Alternatively, calcium influx may control the rate of inactivation of the AH potassium conductance. In the experiments reported here, we also found that theophylline greatly potentiated the calcium-sensitive potassium conductance. Theophylline may increase the stimulus-induced entry of calcium into these neurones, as it does in other tissues^{21,22}, thus augmenting the activation of the potassium conductance; however, we cannot exclude other possibilities, for example that theophylline enhances the calcium sensitivity of the AH potassium conductance.

Theophylline inhibits cyclic nucleotide phosphodiesterases and potentiates cyclic nucleotide-dependent processes in many tissues²³. Cyclic nucleotides may be involved in cellular calcium metabolism^{24,25} and could play a role in the augmentation of the AH by theophylline. In cardiac Purkinje fibres theophylline and cyclic AMP increase both a slow inward current carried by calcium and sodium and a slow outward current carried predominantly by potassium²².

Calcium-sensitive potassium conductances modulate the excitability and the repetitive firing of neurones^{4,10,13,26}. Theophylline may therefore prove to be a useful tool for investigating regulation of neuronal activity and responsiveness.

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Phosphorylation of myelin basic protein by vaccinia virus cores

PROTEIN kinases, catalysing the transfer of the γ phosphate from ATP to serine and threonine residues in protein substrates have been described in several enveloped viruses¹⁻³. Vaccinia virus contains a protein kinase that has recently been well characterised⁴: it is located within the viral core, is not stimulated by cyclic mononucleotides and phosphorylates at least two viral acceptor proteins. The biological functions of the protein kinase and acceptor proteins are unknown, but it is possible that the viral protein kinase could also act on host proteins, thus providing a mechanism by which the virus could modify or control host processes on infection⁴. Vaccinia virus can induce a demyelination after experimental infection, but the mechanism for this is poorly understood⁵. It has been suggested that direct viral action on membranes may have a role in the pathogenesis of virus-induced demyelination⁶. We have therefore investigated the ability of vaccinia virus cores to phosphorylate human myelin membranes in an *in vitro* system and report here that they can phosphorylate purified myelin basic protein as well as basic protein in the myelin sheath.

Purified myelin basic protein was found to be an excellent substrate for the core-associated protein kinase (Figs 1 and 2a). No activator was needed, which suggests that myelin basic protein functions both as an activator and as a substrate⁴. The almost quantitative recovery of the radioactivity from the TCA experiments (Fig. 1) in the peak corresponding to the myelin basic protein on SDS gel (Fig. 2a), shows that the more than tenfold increase in ³²P-incorporation observed with added myelin basic protein, compared with core alone, represents phosphorylation of the myelin basic protein and not an increase in ³²P-incorporation in core proteins. It is possible that part of the phosphorylation of soluble myelin basic protein might actually be phosphorylation of histone contaminants. This seems rather unlikely, however, because the myelin basic protein used migrated as a single band on disc gel electrophoresis at acid pH and radioactivity was found only in the slices corresponding to the myelin basic protein on polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS) (Fig. 2a). The addition of cyclic AMP or 8-bromo cyclic AMP (a cyclic AMP analogue more resistant to phosphodiesterase) did not stimulate the reaction (data not shown). Following a 1-h incubation, a total of 0.5 mol of phosphate was incorporated per mol of myelin basic protein. Determination of the isoelectric point of the myelin basic protein, by extrapolation to zero of the (pH-dependent) mobilities on analytical cellulose acetate sheets¹¹, showed a significant change in the isoelectric point from 10.8 for the native myelin basic protein, to 10.4 for the phosphorylated protein. The phosphorylated fraction was eluted from the cellulose acetate strip and contained 1 mol of phosphate per mol of myelin basic protein.

So far, the demonstration of a direct phosphorylation of host-cell components by a viral protein kinase has been hampered by the high level of endogenous protein kinase activity present in most *in vivo* or *in vitro* systems¹². This endogenous activity

present in myelin membranes in our system can be completely inactivated by mild heating^{10,13}. We have shown that such heated membranes can be used as a substrate for exogenous enzyme¹⁰, thus allowing us to test the ability of the core-associated protein kinase to phosphorylate the myelin basic protein in an environment reflecting the native structural state. Using such heat-inactivated myelin membranes there was a significant increase in ³²P incorporation with added myelin membranes, compared with core alone (Fig. 1). Analysis of the phosphorylated products on SDS-polyacrylamide gel electrophoresis (Fig. 2b) shows incorporation of radioactivity over the band corresponding to the myelin basic protein. It is interesting that the viral protein kinase shows the same specificity as the endogenous protein kinase of myelin¹⁰, because the other major myelin proteins show no incorporation of radioactivity.

We observed a much lower phosphate incorporation with myelin membranes than with isolated myelin basic protein. After incubation for 60 min, 29.7 nmol ³²P-phosphate per mg myelin basic protein was incorporated when isolated myelin basic protein was used as a substrate and 4.25 nmol when myelin membranes were used. Steric factors must therefore play a major part in the incorporation of ³²P, since our system can be compared with a solid-state reaction between the enzyme, bound to the large viral core (molecular weight $\sim 2 \times 10^9$) and the membrane-associated substrate. In the final step of our core preparation non-ionic detergents were removed, to prevent

Fig. 1 Time course of ³²P incorporation with core alone ($\blacktriangle$), core and heat-inactivated myelin membranes ($\blacksquare$) and with core and isolated myelin basic protein ($\bullet$). The reaction mixture⁷, with a final volume of 250 μ l contained 50 mM Tris-HCl, pH 10.25, 5 mM dithiothreitol, 5 mM MgCl₂, 0.5 mM γ -³²P-ATP (30–90 c.p.m. pmol⁻¹), 30 μ g of core protein and either ~ 100 μ g of heat-inactivated myelin protein or 30 μ g of purified myelin basic protein. Incubations were carried out at 34 °C. Reactions were terminated with 2.0 ml of 10% trichloroacetic acid (TCA) containing 0.06 N sulphuric acid. The material was collected on Millipore filters (0.45 μ m), washed extensively, dried and counted in 10 ml of Aquasol in a liquid scintillation counter. Separate blank values, determined for different substrates, were always subtracted. Incubation of heat-inactivated myelin membranes alone for up to 1 h with the total reaction mixture gave the same value as the corresponding zero blank with added enzyme. Vaccinia virus (strain WR) and cores were prepared by slight modifications of the procedure described by Paoletti and Moss⁷. Myelin was prepared from human brain (white matter) by the method of Norton⁸. Heat inactivation was at 55 °C for 5 min and any broken membranes were removed by recentrifuging the heated membranes over a discontinuous sucrose gradient and collecting only the material banding at 0.85 M sucrose. Myelin basic protein was prepared from bovine brain⁹ using Cellex-P column chromatography as a final step.

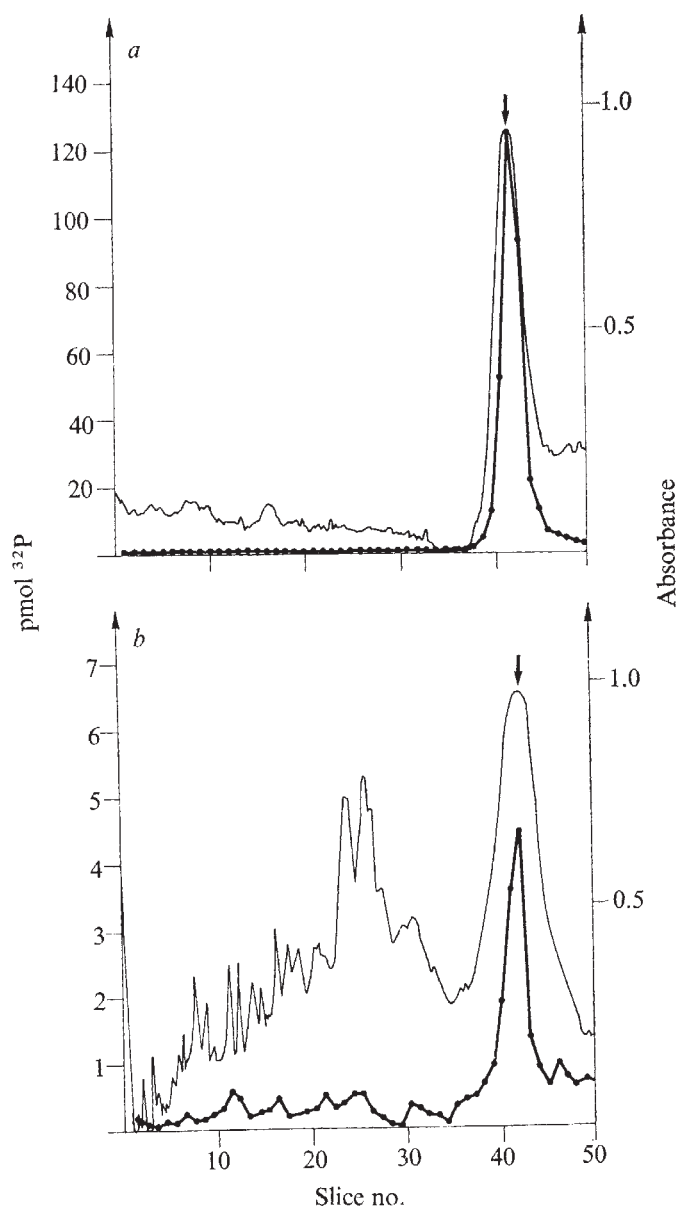
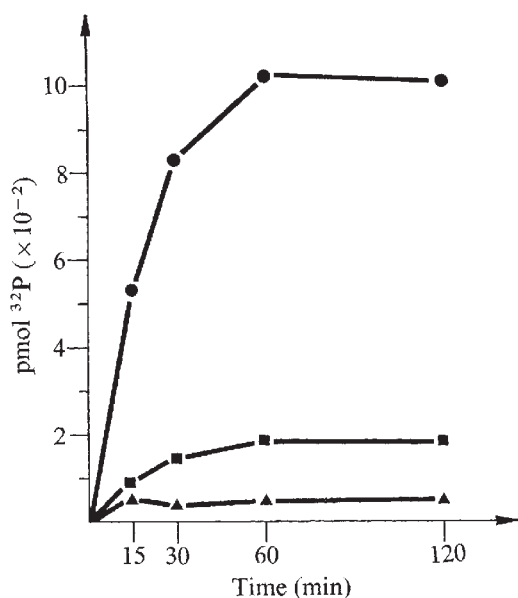


Fig. 2 Absorbance (—) of 7.5% polyacrylamide gel in 0.1% SDS and the corresponding radioactivity of ³²P-phosphorylated products ($\bullet$). Arrow denotes myelin basic protein. The reaction mixture is as described in Fig. 1 but with a final volume of 1,000 μ l; incubation was for 60 min at 34 °C. *a*, Incubation mixture contained 120 μ g of purified myelin basic protein and 120 μ g of core protein. At the end of the incubation, the mixture was cooled on ice, transferred in a centrifugation tube and the core pelleted through 0.85 M sucrose. The supernatant, containing the myelin basic protein, was collected, precipitated with TCA, washed with ethanol-water 9:1 and the resulting pellet solubilised for electrophoresis as described previously¹⁰. About 10 μ g of protein was applied for electrophoresis. *b*, Incubation mixture contained 400 μ g of heat-inactivated myelin membranes (prepared as described in Fig. 1) and 120 μ g of core protein. After incubation the mixture was ice cooled, transferred in a centrifugation tube and the core pelleted through 0.85 M sucrose. The myelin banding at 0.85 M sucrose was collected and solubilised for electrophoresis. About 100 μ g of protein was applied for electrophoresis. Preparation of gels, staining and destaining were as described previously¹⁰. Gels used to measure radioactivity were cut into 2-mm slices and the fractions solubilised in a toluene-based scintillation fluid containing a solubiliser (Protosol). Blank values obtained for the different substrates without enzyme were subtracted. Scanning was at 530 nm using a Zeiss spectrophotometer fitted with a linear transport accessory. The results shown are representative of three different preparations. Note the difference in the scale of the ordinate between *a* and *b*.

any solubilisation of core components. *In vivo*, however, the core dissolves during infection with the subsequent release of its components¹⁴. Very little is known about the fate of many of the viral enzymes, but it has been shown that virus-induced modification of host membranes is not dependent on virus multiplication, host RNA or protein synthesis⁶. It is thus tempting to speculate that phosphorylation of myelin membranes by an enzyme system that is not dependent on endogenous concentration of cyclic nucleotides may occur *in vivo*. This might have a profound effect on myelin structure and function.

As regards the biochemical mechanisms of pathogenesis, the role of the viral protein kinase may involve action on: (1) myelin basic protein, (2) nuclear histones or (3) mitochondrial cytochrome *c*, since the enzyme shows a strong proclivity for basic proteins⁷ and is not specific to myelin basic protein. Whether the phosphorylation of the myelin basic protein, an encephalitogenic protein, by the vaccinia core-associated protein kinase, is relevant to the demyelination observed after experimental vaccinia virus infection remains to be determined.

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Ribonuclear protein formation at locus 2-48 BC in *Drosophila hydei*

In *Drosophila hydei* salivary glands, a puff can be induced by treatment with vitamin B₆ (ref. 1) at locus 2-48 BC. This puff is one of the series that can also be induced by temperature shock, anaerobiosis or treatments interfering with the respiratory metabolism². A product of this puff seems to be a giant ribonucleoprotein (RNP) complex (diameter up to 4,000 Å) with a very specific morphology³. This complex consists of a core and a cortex, formed by a wreath of small particles (diameter approximately 300 Å; ref. 4). Cytochemical analysis has shown that the core of the complex is essentially free of RNA whereas the cortex does contain RNA⁴. Using phospho-tungstic acid (PTA) as a specific stain for proteins, it could also be shown that the proteins from the cortex are relatively rich in arginine and lysine residues whereas those in the core are relatively poor in such residues⁵. No similar puff product has so far been found in any other locus of *D. hydei*. In addition to the giant RNP complexes small RNP particles (diameter up to 300 Å; not to be confused with the particles from

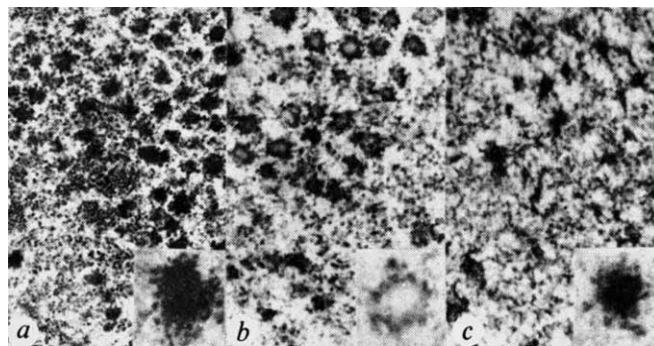


Fig. 1 Staining of RNP from locus 2-48 BC in puff and nucleoplasm (insets). *a*, *b* and *c*, RNP particles and complexes in puff 2-48 BC. Magnifications: *a*, *b* and *c*: $\times 15,360$; insets: $\times 48,000$. Glands were isolated by hand. Puffs were induced by incubation for 3 h in Poels medium containing 5×10^{-2} M vitamin B₆ (ref. 1). After fixation in 3% glutaric aldehyde in sodium cacodylate buffer (pH 7.2) for 30 min at 4 °C, followed by 1 h at room temperature, glands were dehydrated and embedded in Epon⁸, sectioned with an LKB ultratome III and examined using a Philips EM 201. Stainings were as follows: *a*, 3% aqueous uracil acetate for 10 min and subsequently for 0.5–1 min in alkaline lead citrate⁹; *b*, after fixation and dehydration, with 3% PTA in ethanol at 0 °C overnight¹⁰; after washing twice with cold ethanol, glands were embedded and sectioned as described above; *c*, fixation and washing (twice) with distilled water; staining with 5% aqueous PTA (adjusted to pH 1 with concentrated HCl) for 3 h at room temperature (modified from Palladini *et al.*¹¹), followed by three rinses with distilled water, dehydration, embedding and sectioning as described above.

the cortex) can also be seen in this puff^{2,5}. A comparison of data from both ultrastructural and autoradiographical studies on RNA formation at locus 2-48 BC has led to the conclusion that the small RNP particles present in this puff aggregate to the giant RNP complexes and thus give rise to the final puff product⁶. This conclusion is in agreement with the finding of a single RNA species in puffs obtained by microdissection of salivary glands⁷. Although the small RNP particles in the puff have not been studied extensively, it has been assumed that, based on the presence of RNA and arginine and lysine residues, the small particles form the cortex of the complex⁸, leaving the origin of the cortex proteins still obscure. Here I present the results of a comparison between the staining of the small RNP particles with that of the components of the giant complexes. These studies indicate that the same components of the small RNP particle become part of the core of the complex after aggregation.

After induction of puff 2-48 BC with 5×10^{-2} M vitamin B₆, glands were prepared for electron microscopy. A comparison was made between staining with uracil acetate-lead citrate (on grids), PTA in ethanol (on block) and aqueous PTA at pH 1 (on block), (Fig. 1). The results are summarised in Table 1.

It is clear that with all the three staining methods the small RNP particles stain densely. The staining with PTA was independent of the presence of RNA since neither RNase treatment (on block, followed by a mild TCA extraction), nor alkaline (0.1 M NaOH for 24 h at 25 °C) nor acid (TCA 5%, 24 h at 25 °C) hydrolysis markedly affected the staining. Thus the proteins in the small RNP particles in the puff can bind PTA in alcoholic as well as in aqueous solutions at pH 1. In the complex the proteins attached to the RNA in the cortex can still bind PTA in alcoholic solutions but the binding

Table 1 Staining intensity of RNP particles present in locus 2-48 BC

Stain	Small RNP particles	Giant complexes Core	Cortex
Uracil acetate-lead citrate	+++	+++	+++
PTA, alcohol	+++	—	+++
PTA, aqueous	+++	+++	+

capacity for PTA in aqueous solutions is largely lost. The proteins in the core of the complex cannot bind PTA in alcoholic solutions, but do bind, like the small RNP particles, PTA in aqueous solution. From these data it can be inferred that, during aggregation, the small RNP particles lose an aqueous PTA-binding protein that is found later, after aggregation, in the core of the complex. The proteins in the core of the complex must therefore be assumed to derive, at least in part, from the small RNP particles. Other explanations for these observations, such as an exchange of RNP proteins with nucleoplasmic proteins, remain possible but are unnecessarily complicated. Moreover, structures resembling the core of the complex have never been observed separate from the complex. Since, in this kind of study, the available techniques do not permit quantitative analysis on the ultrastructural level, no conclusion can be drawn with respect to the amount of protein involved in the formation of the core of the complex. The presence in the complexes of nucleoplasmic proteins, besides the proteins derived from the RNP particles, cannot be excluded.

Since the giant complexes can be found in a well defined area within the puffed region^{2,5}, an involvement of the genome in the aggregation process could be postulated, but no evidence for such an involvement is available. So far, data concerning a possible function of the aggregation process and the proteins involved in it are also lacking. Such an analysis must await biochemical analysis of isolated complexes. It is not clear to what extent the changes in protein composition in this particular RNP product can serve as a model for RNP formation in general but the formation of this special gene product shows that RNP production can be very complicated, more so than is generally understood from the biochemical data available at this moment.

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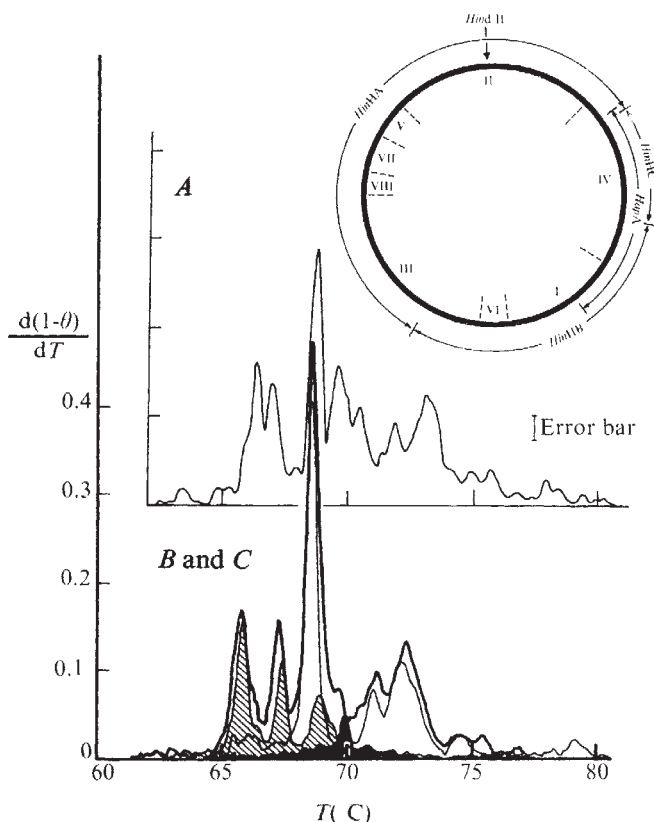
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indication of the second characteristic is that the melting temperature is known to increase with the G+C content. These properties of double-stranded DNA open up a possibility of probing the genetic structure by means of purely physical observations.

A series of studies on the local stability of a DNA has been carried out by a thorough measurement of hyperchromicity caused by the unfolding of the double strand with increasing temperature. Several distinctive features which have been obtained are: (1) Thermal melting is not a continuous process, but consists of a set of discrete steps (refs 1-4 and refs therein). In the case of a relatively short DNA such as λ -DNA (46,000 base pairs), it appears as several sharp peaks over a broad background in a differential display, each of which corresponds to a unit or units of melting having the same stability. (2) Spectral analysis of each peak has shown that the local G+C content in the homostability region is a major determining factor of its melting temperature (C. Akiyama, O.G., and A.W., unpublished). The Marmur-Doty relationship between the melting temperature and G+C content for whole DNA, also holds for localised melting within a DNA molecule. (3) The homostability regions also exist in long DNAs (ref.

Fig. 1 *A*, Melting profile of whole DNA (RF-linear) of fd phage; *B*, after digestion by *RHindII*; that is, a mixture of *HinHA*+*HinHB*+*HinHC* (bold solid line); *C*, profiles of separated fragments, *HinHA* (white area), *HinHB* (hatched area) and *HinHC* (black area), respectively. The scales of the *A* are same as *B* and *C*. Buffer solution: 0.1 $\times$ SSC. Doubly closed replicative form (RF) DNA of fd phage was cleaved by three different restriction enzymes (*RHindII*, *RHinHI*, *RHapII*), and linear RF DNA produced by *RHindII*, three fragments produced by *RHinHI* (*HinHA*, *HinHB*, *HinHC*), and the longest fragment produced by *RHapII* (*HapA*) were prepared as in ref. 5. Top right, the *HindII* cleavage site and region covered by each fragment are indicated together with an approximate location and size of fd genes⁶⁻⁸. (Roman numerals indicate the gene number.) Melting profiles were obtained with a minicomputer controlled on-lined melting plotter⁹. The displays are made in the differential form $d(1-\theta)/dT$ against T , where θ is helix content, T temperature, and $(1-\theta)$ for the fragments is defined as $(1-\theta) = (1-\theta) \times (\text{length of fragment/length of whole DNA})$.



Long range homogeneity of physical stability in double-stranded DNA

WE present here evidence for the existence of regions with homogeneous physical stability (homostability regions) in double-stranded DNAs. Results of a study on the thermal melting of whole replicative form DNA from fd phage and of its fragments obtained by the action of restriction enzymes, strongly suggest that regional homostability seems to be an intrinsic characteristic of DNA, playing an essential part in its genetic activities.

A sequence of A-T and G-C paired bases that stabilises two nucleotide chains into one double-helical conformation is responsible for both the biological and physical characteristics of DNA; that is, the genetic information is 'written' by a variation of that sequence on the one hand, and the physical stability of the double-stranded structure is determined by the base composition on the other hand. An

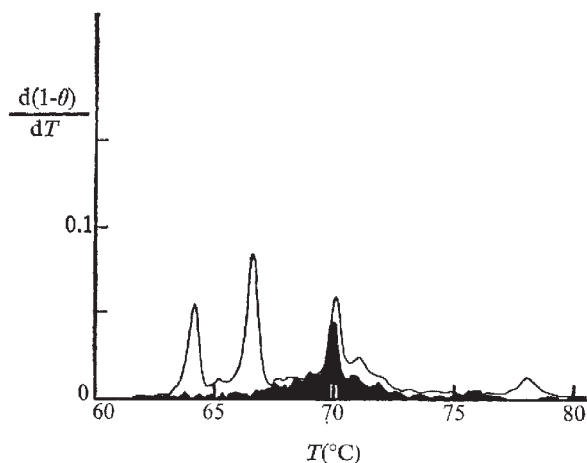


Fig. 2 Melting profiles of *HapA* (○), and of *HinHC* (●) fragment which is totally involved in *HapA*.

4 and O.G., S. Yabuki and A.W., unpublished), although the fine structures in the melting profile become obscure, probably because of random overlapping of the clustered peaks. The existence of the homostability region seems to be a general attribute of the DNA. (4) Studies on the DNA from deletion mutants have shown that a single peak in the melting profile can result from the melting of one or several homostability regions^{2,3}. (5) From a computer simulation for model DNAs of given sequences³, it is strongly suggested that a long range homogeneity in the base sequence (>500 base pairs) is necessary to produce a single sharp peak similar to those observed in our experiment. Here "homogeneity" does not mean either a regularly repeated sequence or homopolymers, but refers to a fairly long sequence consisting of over 500 base pairs in which A-T and G-C pair sequences alternate at frequent intervals made up of no more than a few base pairs³.

In addition to the facts mentioned already, much new evidence, obtained with a much shorter DNA from fd phage and its fragments made by restriction enzymes³, have made the characteristics of regional homostability much clearer.

The most distinctive feature of the differential melting profile of the whole fd-DNA (RF-linear; 6,000 base pairs) is that all the peaks are very sharp and well separated from one another (Fig. 1A). By the action of the restriction enzyme *RHnHI* on the DNA (RF-circular), the melting profile changes to yield a set of different, but still sharp and well separated peaks (Fig. 1B, bold line). The individual melting profiles of each digestion product, *HinHA* (~3,500 base pairs), *HinHB* (~1,800 base pairs), and *HinHC* (~700 base pairs), were measured after separation by gel electrophoresis, and are shown together in Fig. 1C. The genetic map is also shown, to allow location of the fragments in it⁶⁻⁸. These three digestion product melting profiles, when summed, are found to agree with the profile of the mixed DNA (Fig. 1B). There are, however, not quite additive enough to reproduce that of the whole DNA (RF-linear) (Fig. 1A). Figure 2 shows the melting profile of *HapA*, the fragment produced by a different digestion process, compared with *HinHC*, and from the map shown in Fig. 1, *HinHC* is totally involved in *HapA*.

These experimental results from the fragments indicate that the homostability region is not uniquely defined by short range stability of the double-stranded DNA. A long range interference between the homostability regions may come through the loop entropy and electrostatic interaction of the melted region between them.

From the results, together with the genetic map, we may now conclude the following. (1) The DNA is found to consist of a number of homostability regions which come from

homogeneous base sequences consisting of 500 base pairs or more. (2) One genetic unit (the operon) may contain one or a few homostability regions. (3) Sometimes different genetic units have homostability regions with the same degree of stability. (4) A homostability region exerts the influence of the melting to other regions away from it, which sometimes produces splitting or coalescence of peaks. For instance, the highest peak of *HinHA* splits into two peaks in the profile of whole DNA, probably because of cleavage by *RHindII*.

Location of the homostability regions on the DNA and the elucidation of the mechanism of interactions among these regions are an interesting subject for the study of the statistical mechanics of DNA chains.

Biologically, it is hard, if not impossible, to believe that such regional homostability originates in a fundamental characteristic of the genetic code itself. It is quite plausible, however, that the homostability region plays an important part somewhere in the biological process within which the DNA is closely related. If so, then the evolutionary selective force can be considered to have fixed such regions in DNA. From the size of the homostability region, recombination⁹ might be one possible process which is aided by it. In any case, the wobble bases must give the necessary redundancy to make a homostability region without spoiling the biological meaning of the genetic code: the activity of proteins.

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Specific cleavage of chloroplast DNA from higher plants by *EcoRI* restriction nuclease

THE physicochemical properties of chloroplast (cp)-DNA in higher plants have been reported¹⁻⁴. Cp-DNAs occur as closed circles of molecular weight 85×10^6 – 97×10^6 . Specific identification of cp-DNAs isolated from different plants cannot, however, be carried out on the basis of buoyant density, melting points, kinetic complexity or direct length measurement by electron microscopy, since each of these techniques give values which fall within narrow limits, and only overlap with one another a little^{1,2}.

The analysis of restriction nuclease digests by gel electrophoresis provides a very sensitive test to compare small DNA molecule species³. This technique has been applied successfully to various animal mitochondrial DNAs⁶ and to mt-DNA of wild and petite yeasts⁷. We show here that

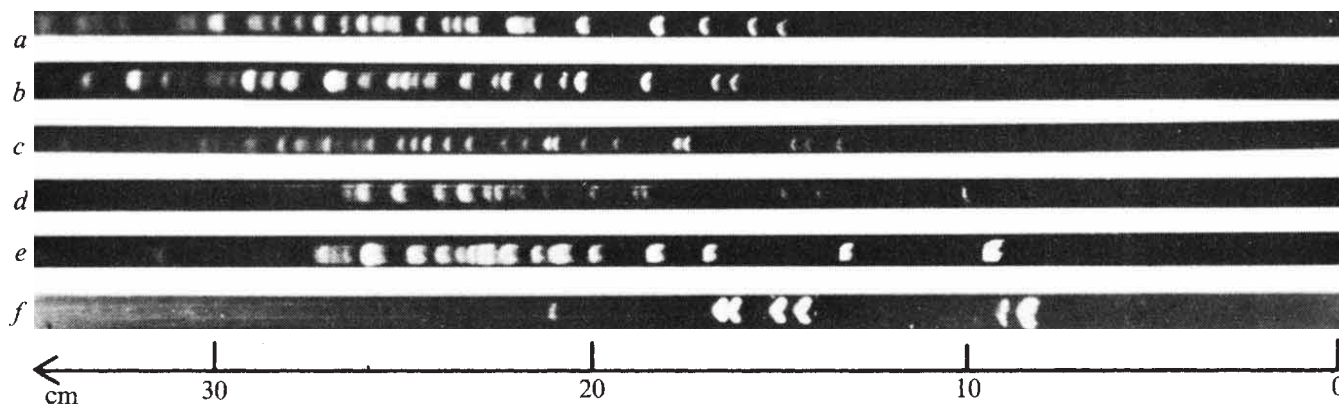


Fig. 1 Agarose gel electrophoresis of *EcoRI* digest of cp-DNAs from leaves of: *a*, pea; *b*, spinach; *c*, tobacco, *N. tabacum* var. Samsun; *d*, maize; and *e*, wheat. λ plac5 DNA digest (*f*) was used as a standard for molecular weight determination. The arrow indicates the direction of electrophoresis. Isolation of chloroplasts, DNA extraction from DNase-treated organelles and two-step CsCl ethidium bromide (or propidium di-iodide) gradient centrifugation of cp-DNA have been described previously². The drug was removed using freshly redistilled butanol saturated with 0.01 M EDTA pH 8. DNA samples were then dialysed against three 500-ml changes of 0.1 M NaCl, 0.05 M Tris, 0.01 M EDTA pH 8.0. The cp-DNAs were pelleted at 35,000 r.p.m. for 10 h in a SW41 rotor after adjusting the NaCl concentration to 1 M. cp-DNA pellets were then dissolved in 0.1 M Tris, 0.001 M EDTA pH 7.5 and cleared by centrifuging for 30 s at 20,000g. The yields were about 30–50 μ g of supercoiled cp-DNA per 100 g of leaves, depending on the plant species. DNA samples were analysed for purity and concentration by analytical CsCl gradient and were checked for circularity by electron microscopy⁹. To 20 μ l of purified cp-DNA (2–3 μ g DNA) were added 20 μ l of 0.1 M Tris, 0.03 M $MgSO_4$ pH 7.5 and 5 μ l of *EcoRI* endonuclease (enough enzyme to give a limit digest of 10 μ g of λ DNA). Digestion was carried out at 37 °C for 3 h. The reaction was stopped by addition of EDTA to a final concentration of 15 mM and cooling to 4 °C. Each sample was treated with one volume of water-saturated phenol. Solid sucrose was added up to 20% and samples were layered on top of cylindrical 0.7% Agarose gels (type I, Sigma), 8 mm in diameter and 35 cm in length. Electrophoresis was carried out in 0.05 M Tris, 0.02 M Na-acetate, 0.002 M EDTA pH 8.05 (with glacial acetic acid) plus 0.18 M NaCl (ref. 5) at 100 V for 15 h, by which time, the bromophenol blue marker is ~4 cm from the bottom of the gel. Gels were extruded gently from the plastic tubes and stained in darkness for 2 h in the electrophoresis buffer supplemented with 1 μ g ml⁻¹ ethidium bromide. They were then layered on a black background, illuminated at 254 nm with a Camag TL 900 ultraviolet lamp and photographed through an orange glass filter (Wratten 77 A) on Kodak Tri X 35-mm film. Microdol X was used for development (10 min).

cp-DNA extracted from different higher plants can be characterised and distinguished easily by gel electrophoresis of *EcoRI* digests.

Pisum sativum var. très hatif Annonay nain, *Spinacia oleracea* var. Monstrueux de Viroflay, *Nicotiana rustica* var. Pavonii, *Nicotiana tabacum* var. Samsun, *Nicotiana glauca*, *Zea mays* var. INRA 200, *Triticum aestivum* var. Capitole, were grown in a greenhouse. Chloroplasts were extracted from leaves (deribbed for spinach and tobacco) and cp-DNA isolated from DNase-treated cp-pellet according to a procedure already described², except that the two-step differential centrifugation used in organelle purification was repeated. The supercoiled cp-DNA was isolated as the lower ultraviolet fluorescent band of ethidium bromide (or propidium di-iodide) CsCl gradients. cp-DNAs were concentrated by pelleting after removal of drug and the purity was checked by analytical CsCl gradients (unimodal distribution). Control of circularity was achieved by Kleinschmidt spreading and positive staining with acid-alcoholic uranyl acetate⁸. The purified cp-DNAs were then digested by *EcoRI* endonuclease and the digest analysed by electrophoresis on 0.7% Agarose cylindrical gels. The *EcoRI* restriction fragments of λ plac5 DNA were used as mol-

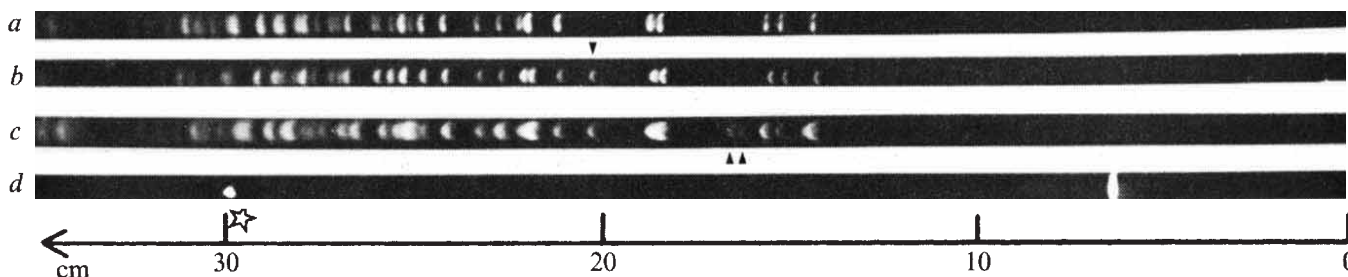
ecular weight standards¹. Gels were stained with ethidium bromide and ultraviolet fluorescence photographs were taken.

Figure 1 shows the electrophoretic pattern of the cp-DNAs isolated from pea, spinach, tobacco (*N. tabacum* var. Samsun), maize and wheat. Some 30 distinct bands were resolved in each case, about 25 moving more slowly than, or with, the bromophenol dye. The same number of bands was obtained whether electrophoresis was achieved on 35-cm or 60-cm Agarose gels. The cp-DNA of a given plant seems to be characterised by a specific digestion pattern and so may be easily distinguished from that of other plants belonging to different genera.

The characteristic band patterns of various cp-DNAs isolated in this way from several higher plants were found to be easily reproducible. For example, several independent preparations of cp-DNA have been made for each plant mentioned above. Separate digestions of these cp-DNAs were performed and the resulting band patterns found to be invariant. Identical patterns were obtained for a given plant with incubation times of cp-DNA in the presence of the *EcoRI* enzyme ranging from 1 h to 6 or 12 h.

The molecular weights of *EcoRI* fragments from

Fig. 2 Agarose gel electrophoresis of *EcoRI* digests of cp-DNAs from deribbed leaves of *a*, *Nicotiana rustica* var. Pavonii; *b*, *Nicotiana tabacum* var. Samsun; *c*, *Nicotiana glauca*; *d*, native cp-DNA of *Nicotiana tabacum* var. Samsun, a small spot (Star) of ethidium bromide was superimposed on the bromophenol blue before taking the ultraviolet photograph. Arrow indicates the direction of electrophoresis. Isolation of cp-DNAs, endonuclease digestion and electrophoresis of DNA fragments were performed as described in Fig. 1.



cp-DNAs shown in Fig. 1 have been determined from both their mobilities relative to λ plac5 *EcoRI* fragments and from their length as measured by electron microscopy after gel slicing and DNA recovery. The sum of the band sizes for each electrophoretogram of the Fig. 1 is 38×10^6 – 45×10^6 daltons, according to the plant, the monocots giving the lowest values. Such molecular weights represent only about half the molecular weight already estimated from the contour length of the native cp-DNA molecules and from the reassociation kinetics^{1,2}; monocots displaying the lowest values here also. The fluorescence intensity of the bands does not decrease steadily with decreasing molecular weight fragments. Some bands exhibit a fluorescence intensity higher than expected and it is likely that these bands are multiple. Experiments with radioactive cp-DNA are required to state the band multiplicity precisely.

The characteristic band patterns produced after *EcoRI* digestion of cp-DNA from three plants of the genus *Nicotiana* (*N. rustica* var. Pavonii, *N. tabacum* var. Samsun, *N. glauca*) are presented on Fig. 2. It is noticeable that the three patterns are very similar, although not identical. A difference involving a few bands (indicated on Fig. 2) allows the recognition of each *Nicotiana* species. Gel electrophoresis of *EcoRI* digests provides an extremely sensitive method to evidence intergenera as well as intragenus differences at the cp-DNA level.

This method should be very useful to (1) demonstrate the function of cp-DNA by illustrating the association of genetic markers with individual groups of restriction fragments; (2) confirm the nature of parasexual hybrids; (3) analyse the phylogeny within some important genera; and (4) elucidate the origin of male sterile line.

We thank Dr H. Fukuhara for a gift of *EcoRI* endonuclease and Dr Tiollais for the λ plac5 DNA used in these studies.

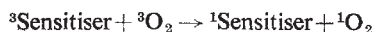
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reaction⁵) or by energy transfer from triplet states of sensitiser according to the following scheme²

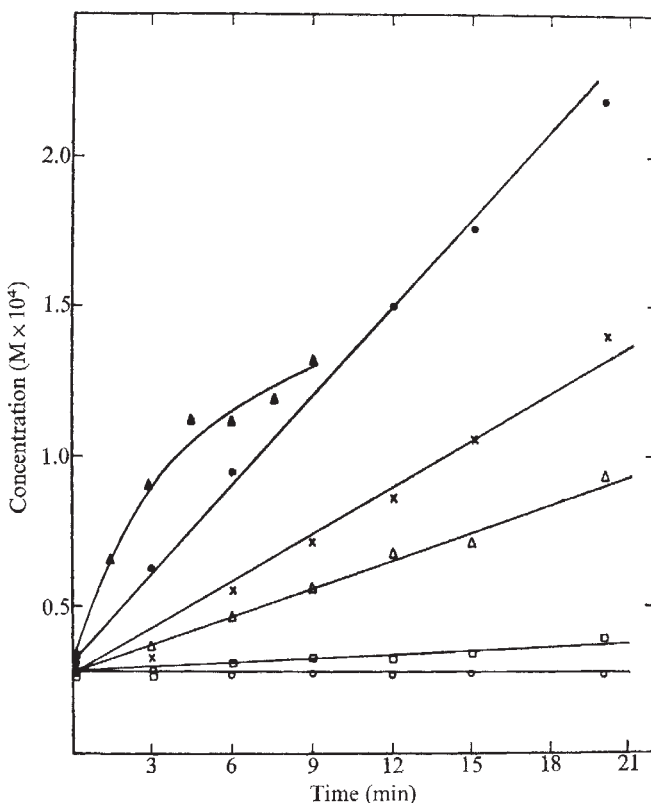


Singlet oxygen can be identified as an intermediate in a given reaction by (1) the quenching effect of the azide ion ($k_q = 2.2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ in methanol⁶); (2) the enhancing effect of deuterated solvents⁷; (3) the identity of the oxidation products of a substrate to those obtained in a reaction known to involve singlet oxygen⁸.

Quenchers other than NaN_3 have also been studied. Among these, amines constitute a separate class, probably because they are highly specific for the $^1\Delta_g$ state³. Typically, their quenching rate constant (k_q) ranges from 10^6 – $10^7 \text{ M}^{-1} \text{ s}^{-1}$ (ref. 8). The quenching mechanism has not been fully elucidated, however. The existence of a charge transfer complex between the amine and the singlet oxygen molecules has been proposed; the rationale for such a hypothesis is the good correlation found between $\log k_q$ and the ionisation energy of the amines⁸. Since such complexes are reversible, the amines are usually considered as inert quenchers³. A fairly new class of stable free radicals, the nitroxides, are, however, commonly synthesised by oxidation of sterically hindered amines⁹. The high specificity of amines for $^1\Delta_g$ and the possibility in some particular cases of stabilising the oxidation products, led us to try a new method in which stable nitroxide radicals would be generated by reaction with singlet oxygen and would be easily detectable by electron spin resonance (ESR) spectroscopy.

In our experiments, singlet oxygen was produced either through the classical chemical reaction between H_2O_2 and NaOCl or by energy transfer, and 2,2,6,6-tetramethylpiperidin was added to the medium. A slight ESR signal was already

Fig. 1 Nitroxide concentration against duration of light exposure Δ , Proflavin; $\bullet$, toluidine blue; $\times$, fluorescein; $\triangle$, retinal; $\square$, xanthoxin; $\circ$, without sensitiser. Sensitiser concentration, 10^{-3} M ; 2,2,6,6-tetramethylpiperidin concentration, $1.15 \times 10^{-1} \text{ M}$; nitroxide concentration before illumination, $0.3 \times 10^{-4} \text{ M}$. Light source, Osram HBO 500; filters, Schott WG 345 and KG1; total incident intensity (300–800 nm): $14 \times 10^{18} \text{ photons cm}^{-2} \text{ s}^{-1}$ (by chemical actinometry).



New method of detecting singlet oxygen production

SINGLET oxygen is receiving increasing attention as a reactive species in many chemical or biological reactions (see refs 1–3 for recent reviews). We report here a new method for detecting the formation of singlet oxygen by the generation of stable nitroxide radicals from sterically hindered amines.

Excited oxygen (singlet oxygen) can be produced for example, by microwave discharge in the gaseous phase⁴, by chemical reactions (the first reported one being the hypochlorite– H_2O_2

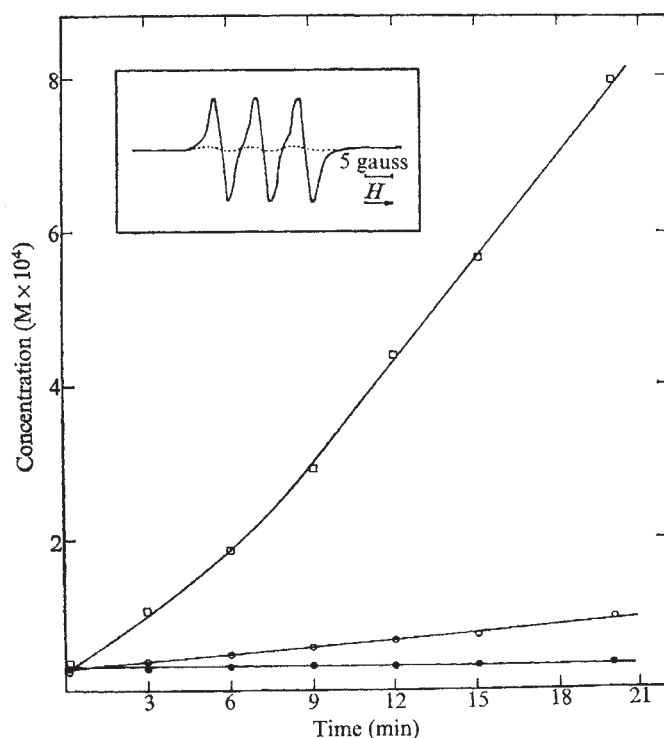


Fig. 2 Nitroxide concentration against duration of light exposure in the presence of 10^{-3} M retinal. 2,2,6,6-tetramethylpiperidin concentration 1.15×10^{-1} M; nitroxide concentration before illumination, 0.3×10^{-4} M. □, $\text{CD}_3\text{CD}_2\text{OD}$; ○, $\text{CH}_3\text{CH}_2\text{OH}$; ●, $\text{CH}_3\text{CH}_2\text{OH} + \text{NaN}_3$ (concentration, 10^{-2} M). Illumination conditions are the same as in Fig. 1. The lag period observed in deuterated ethanol can be interpreted in terms of a buildup period for singlet oxygen steady-state concentration. Inset: ESR spectra before (...) and after (—) illumination. Microwave power 4 mW; modulation amplitude 1 gauss; temperature 22°C .

observed before any reaction. This spectrum characterises nitroxide free radical¹⁰ present as an impurity in the commercial product (Aldrich); its concentration does not exceed 0.3%.

When the chemical reaction is performed in the presence of the amine, free radical production occurs. The corresponding ESR spectrum is identical to the one observed before reaction but its intensity is greatly increased. This suggests that it is the presence of singlet oxygen which leads to nitroxide formation.

For testing the photochemical process, various sensitizers were studied. Toluidine blue and fluorescein are known to generate $^1\text{O}_2$ (refs 11 and 12). It has only been suggested that retinal and proflavin are able to do so^{13,14}. On the other hand, although there are some indications¹⁵ that furocoumarins do not react with oxygen in biological sensitizations Poppe and Grossweiner recently¹⁶ attributed lysozyme photodynamic inactivation to singlet oxygen generated by energy transfer from xanthotoxin triplet state; this latter compound was therefore studied.

Photochemical experiments were performed mainly in ethanol. One millilitre of sensitizer solution (1 mM) was introduced into rectangular quartz cuvettes (1 × 1 cm) and 20 μl of amine were added. Aliquots were taken in calibrated capillaries (Drummond microcaps 50 μl) before exposure to light and after different exposure times. Samples were immediately measured with a Varian E3 ESR spectrometer.

Illumination of the different chromophores also led to nitroxide production. Their rate of formation is shown in Fig. 1. In our experimental conditions, proflavin gave rise to the steepest initial slope but underwent rapid auto-oxidation. Toluidine blue, on the other hand, gave a fairly linear yield up to a 20-min exposure. Therefore it seems to be a very good sensitizer for nitroxide photoproduction, comparable to

retinal and fluorescein, although less effective. With respect to xanthotoxin, the small but measurable yield observed seems to confirm the results reported by Poppe and Grossweiner¹⁶.

To prove definitively that singlet oxygen is implicated, the effects of sodium azide and deuterated ethanol were studied and the results are presented in Fig. 2. In deuterated ethanol, the free radical rate formation increases more than tenfold whereas in the presence of azide, free radical production is abolished.

When experiments were carried out in nitrogen-bubbled solutions, no nitroxide formation occurred. The illumination was usually carried out in an open cell in equilibrium with the atmosphere. In those conditions, the ESR linewidths are fairly large (2.25 gauss) due to the high oxygen concentration in ethanol (6.4 mM). When experiments were performed in a sealed cell, an important linewidth reduction was observed during the photochemical reaction. This phenomenon reflects oxygen consumption within the medium.

In conclusion, a new method for detecting singlet oxygen production can be derived from the experiments reported here. The results shown in Fig. 2 indicate that the reaction is highly specific for singlet oxygen. The technique allows a specific study of free radical formation without interference from other possible side products. Moreover the method, which is convenient and easily set up, can detect free radicals at concentrations as low as 100 nM. Preliminary results performed in aqueous solutions gave similar results to those obtained in ethanol. Possible applications of the method to biological reactions have still to be explored.

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Triplet state involvement in primary photochemistry of photosynthetic photosystem II

THE primary photochemical reaction of photosynthesis is widely accepted to be a one-electron transfer from a chlorophyll (or bacteriochlorophyll) species to an acceptor entity¹. Until recently it had been assumed that the excited state precursor to the electron-transfer reaction was a singlet state in each of the photosynthetic photosystems. This view is based on the fact that estimated fluorescence lifetimes² of ~ 7 ps at the reaction centre are much too short compared with intersystem crossing times of the order of 5 ns for chlorophyll *in vitro*³. Robinson⁴ pointed out, however, that the increase in fluorescence yield observed when the photosystem II (PS II) trap is closed is far less than that expected if photochemistry, such as electron transfer, were the sole quenching process. He and others^{4,5} have proposed the involvement of a triplet state in the mechanism of the photochemical electron transfer, but this

suggestion has received little attention. Blankenship *et al.*⁶ reported an electron spin resonance (ESR) signal arising from photosystem I (PS I) which initially exhibited microwave emission due to chemically induced dynamic electron polarisation (CIDEP). They maintained that this observation indicated that the excited state precursor to the formation of the radical species must have been in a triplet state to account for the CIDEP phenomenon. We report here the first observation of ESR signals exhibiting the CIDEP phenomenon from free radicals associated with PS II of chloroplasts and whole algae. As discussed below, this provides additional evidence that triplet states may be involved as intermediates in the mechanism of photochemical electron transfer in photosynthesis.

In our experiments we used a Varian E12 ESR spectrometer with the addition of a flash-photolysis excitation and detection system⁷, a Photochemical Research Associates Model 610 flash system with a flash half life of $\sim 25 \mu\text{s}$ and a Biomation Model 610B Transient Recorder buffered into a Fabritek Model 1072 Instrument Computer. The flashes were filtered by a Corning CS2-64 red filter. We modified the response time of the ESR spectrometer so that the rise time was $50 \mu\text{s}$ using 100-kHz field modulation and have observed kinetic responses at temperatures from 10 K to 300 K in the whole algal systems.

Figure 1a shows a typical kinetic response for *Scenedesmus obliquus* algae grown on a 99% deuterated medium⁸. We have also obtained responses from spinach chloroplasts, but signals from the whole algae *Anacystis nidulans* and *Scenedesmus obliquus* were more intense and more reversible than signals obtained from the chloroplasts. A negative excursion in Fig. 1a corresponds to the microwave emission mode and a positive excursion to the microwave absorption mode of ESR. If the photochemistry of PS II is blocked, by means of several red flashes before freezing the algae in the presence of 0.1 mM hydroxylamine and 20 μM DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea)⁹, the kinetic response is greatly diminished (Fig. 1b). This, and further evidence given below, leads us to believe that the detected ESR signals arise from free radicals associated with PS II. The signal shown in Fig. 1a was reproducible from the same sample for many thousands of light flashes.

ESR spectra at a fixed time after the flash were obtained from kinetic traces taken at many magnetic field positions close to $g = 2$. It was apparent in spectra from normal algae grown on protonated media that two separate fast transients gave rise to superimposed spectra in the free electron region. Furthermore,

Fig. 1 A typical flash-induced ESR kinetic profile for fully deuterated *Scenedesmus obliquus* frozen as a thick suspension in 20 mM phosphate buffer (pH = 7.5) with 10 mM NaCl and 50% glycerol at 10 K with 0.1 mW microwave power. Collection of 128 flashes in the presence of 20 μM DCMU and 100 μM hydroxylamine: a, after freezing in the dark; b, after freezing following several red flashes at 300 K.

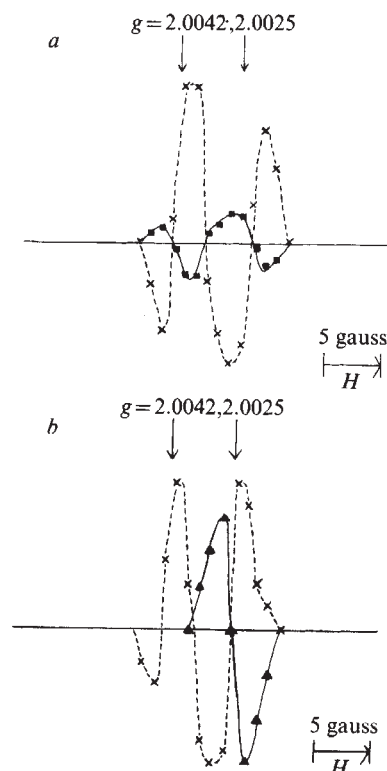
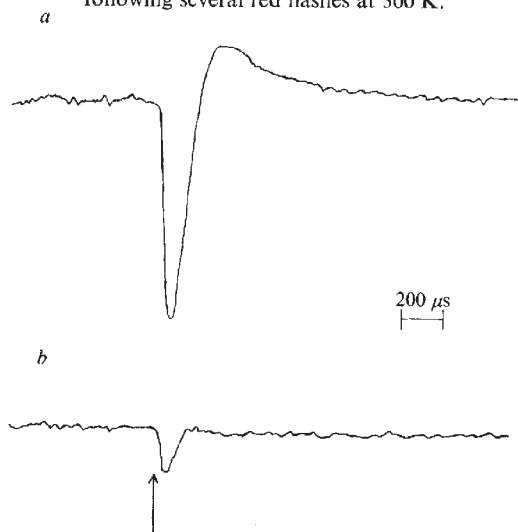


Fig. 2 a, A point-by-point magnetic field profile of the rapid transient amplitudes ($\times$) and of the slow transient amplitudes ($\blacksquare$) induced by flashing 256 times fully deuterated *Scenedesmus obliquus* suspended in 20 mM phosphate buffer (pH = 7.5) with 10 mM NaCl and 50% glycerol at 100 K with 1 mW microwave power and 2.5 gauss modulation amplitude at 100 kHz. The g factors are accurate to a precision of ± 0.0003 . b, A point-by-point field profile of the rapid transient amplitudes ($\times$) and of transient amplitudes of Signal 1 ($\blacktriangle$) induced by flashing 1,024 times fully deuterated *Scenedesmus obliquus* suspended in the same medium at 200 K with the same instrumental conditions and precision as in a.

the observed spectral line shapes for the fast transients were mixtures of absorption and dispersion signals arising, we believe, from rapid passage effects¹⁰. The spectra of rapid and slow transients (Fig. 2a) for deuterated algae, however, were found to be simpler because of less overlapping of the spectra of the separate transients as a result of the collapse of some unresolved proton hyperfine structure. Rapid passage effects are also responsible for some distortion of the line shapes in Fig. 2a.

Figure 2b shows the field profile of the fast transients for deuterated algae relative to the reversible part of P700 (signal 1) at a temperature of 200 K. It is evident that the fast transient signal is in opposite phase to that of signal 1, but nonetheless, it is readily detectable at this temperature. It is significant that the new transient is detectable at temperatures down to 10 K, where signal 1 is not significantly reversible for detection on a millisecond time scale. This behaviour implies that the reversible transient signal is independent of the primary photochemistry of PS I.

We interpret the kinetic response in Fig. 1a to fast and slow decay processes in opposite phase. The fast decay corresponds to electron spin lattice relaxation causing the spin populations (initially inverted) to relax to a Boltzmann distribution. The slow decay corresponds to a chemical disappearance of the radicals. On the basis of the spectra observed for the rapid transients from both protonated and deuterated algae, we can assign the two observed radical species, one with $g = 2.0042$ and another with $g = 2.0025$. The latter corresponds very well to the ESR spectrum of P680⁺, the chlorophyll *a* species acting as the primary donor of PS II and the former could arise from a

plastoquinone anion radical. There is now good evidence for P680 being the primary electron donor¹¹⁻²⁴ in PS II, and there is good optical evidence that a plastoquinone molecule acts as the primary acceptor²¹⁻²⁶, although no one to date has detected an ESR signal from the primary acceptor. The slow phase of decay of Figs 1a and 2a could correspond to an electron-transfer back reaction between the primary donor and acceptor. A decay half time of ~ 1 ms for both the donor and the acceptor is comparable with the 3-ms decay measured optically at low temperatures in chloroplasts²¹⁻²⁴.

Given that our interpretation of the kinetic response and ESR spectra is correct, we should explain how a population inversion of spin states might be established when the free radicals are created by the photochemical electron transfer from P680 to the primary acceptor. The phenomenon of CIDEP was first observed²⁷ by Fessenden and Schuler and has since been observed for many radicals formed in solution²⁸. As yet, the work of Blankenship *et al.*⁶ and our present work are the only reports of CIDEP from a biological solid-state system.

Two mechanisms have been proposed²⁸ to explain the observation of CIDEP. The first is the radical pair mechanism, but it can be excluded in the case of a solid-state system, because it requires free diffusion of radicals in a dynamic fluid state. The photochemical triplet mechanism involves an intersystem crossing into a triplet excited state with unequal rates of populating the three triplet levels. If electron transfer occurs before spin-lattice relaxation in the triplet state can restore a Boltzmann equilibrium, then both the resulting radicals should be observed with initially inverted spin populations. Clearly, the triplet mechanism can explain our results since we have a solid-state system.

If a triplet state of the P680 chlorophyll species is the precursor to photochemical electron transfer in PS II, then intersystem crossing must occur very quickly. Fluorescence decay measurements²⁹ indicate that fluorescence t_1 are no more than ~ 100 ps in PS II, which suggests that there must be some mechanism for enhancement of the intersystem crossing rate to allow for such rapid quenching of fluorescence. One possibility is that the manganese present in PS II is in close proximity to P680: transition metal ions are known to enhance intersystem crossing rates³⁰. One other possibility is that P680 is a dimer of chlorophyll *a* which has a structure analogous to that of P700 in PS I. The quenching of the excited singlet state would then correspond to a charge separation within the dimer pair (P^+P^-). In such a charge-transfer state, the separation between singlet and triplet states would be small (because of the greater distance between the two unpaired electrons), and so an enhanced intersystem crossing rate would be expected.

Our results do not prove that a triplet state must be an intermediate in the photochemistry of PS II, as there may be another mechanism which could produce the CIDEP phenomenon from a single-state precursor, but they should provide impetus to a search for evidence of short lived triplet states in photosynthetic systems, using picosecond optical spectrometers.

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Fluorescence lifetimes of haem proteins excited into the tryptophan absorption band with synchrotron radiation

FLUORESCENT spectra of tryptophan residues in proteins are a powerful tool in the study of the intraprotein medium, primarily as an indicator of local conformational changes of the residue microenvironment. Fluorescence quantum yields of tryptophan in the case of haem proteins, however, are very small ($Q \leq 0.002$)¹, and a dramatic increase ($Q' \sim 0.2$) is observed after haem removal. The photodissociation of carbon monoxymyoglobin, when irradiated using ultraviolet light, has indicated the transfer of excitation from the aromatic amino acid to the haem group^{2,3} following a mechanism of the Förster type⁴; this transfer has a probability¹ at least 100 times greater than the fluorescence, and therefore some 20 times greater than the other competing radiation-free processes. Consequently lifetimes have been considered to be extremely small and no attempts have therefore been made to measure them. This type of information is, however, vital for our understanding of the quenching mechanism by haem. We have therefore reinvestigated the problem with respect to decay times for ox, horse and adult human haemoglobins and their α and β subunits when excited in the tryptophan absorption band. Our data are not refined—lifetime values reported here have a rather poor precision ($\sim \pm 0.25$ ns)—but those obtained using ox, horse and human samples demonstrate similar behaviour. In some selected cases data from haemoglobin and apohaemoglobin have been compared.

Membrane-free haemoglobin samples were prepared by 22,000 r.p.m. centrifugation⁵. The haemoglobin solutions were (poly)crystallised according to Drabkin's method⁶, and fresh samples were used. Haem-free haemoglobin samples were obtained from human adult haemoglobin by precipitation in acid acetone medium and then renatured as described previously⁷.

Haemoglobin splitting in corresponding subunits was carried out according to Bucci's procedure⁸; thiol group regeneration, following Geraci⁹; and free SH titration in accordance with Boyer¹⁰. The purity and the homogeneity of α_{SH} and β_{SH} chains was controlled by electrophoretic analysis over starch gel, and by optical absorption spectroscopy. Their non-denatured character was verified by determining their capacity of association for haemoglobin reconstitution.

Ferrihaemoglobin was prepared by oxidation of deoxy-haemoglobin *in vacuo* with potassium ferricyanide in moderate (5-10%) excess ($pH \sim 7$), the ferricyanide surplus being eliminated by gel filtration through a G25 Sephadex column in phosphate medium.

For all samples a common solvent was used: $10^{-2}M$ KH_2PO_4 - Na_2HPO_4 ($pH=7$) buffer in sterile and clear commercial Biosedra water, presenting a weak fluorescence. The pre-existing organic phosphates (DPG) in the haemolysate have not been eliminated, their concentration in the stock solution being estimated¹¹ at between 0.7 and 1 equivalent per tetramer of

protein. Carc was taken to avoid bacterial contamination (filtration through adequately treated 0.2- μ m Millipore).

Native protein solutions (about 2.10^{-6} M, of haem or of polypeptide chain) from three different donors of each mammalian species (four in the human case) have been studied at $25 \pm 0.5^\circ\text{C}$ and $3 \pm 0.5^\circ\text{C}$, always with fresh samples not older than 15 h, and stored at 4°C in the above buffer.

Fluorescence decay times were obtained using the synchrotron radiation from the Orsay Storage Ring (ACO; for a technical description of the ACO machine, see Fourth, Fifth and Sixth int. Conf. High Energy Accel., Dubna (1963), Frascati (1965) and Cambridge, Massachusetts (1967) and ref. 12.) as a pulsed light source coupled with the Single Photoelectron Timing Method^{13,14}. The ACO machine was working in the e^+ , e^- collision ring mode for a high energy physics experiment with 510-MeV electron energy and 25–10-mA electron circulating current; in these 'parasitic' conditions the available light intensity is smaller by a factor of 5–10, compared with the photon flux obtained using ACO working in the Storage Ring mode, fully geared to synchrotron radiation.

Samples were excited at three different wavelengths (270, 280 and 290 nm), selected using a high flux vertical 'Lavollée-type' monochromator¹⁵, the excitation spectral bandwidth being about 32 Å. All the emitted fluorescence with wavelength higher than 340 nm, was detected at right angles through a set of three Schott-type filters (WG 320, 330 and 345) using a RTC 56 DUVP/03 photomultiplier. Decay curves were recorded by counting signals, in the positive mode for a given time, from the excited haemoprotein solution; and then, in the negative mode, signals for an equivalent active time from the buffer solvent at the same excitation wavelength. With this latter operating mode (which is justified by the fact that synchrotron radiation is perfectly constant in shape and amplitude), and because of the small optical density of the haemoprotein solutions (see below), the stray light level from the excitation is minimised. Therefore, when verified using a MgO diffusing screen, it turns out to be less than 1%, in any case.

We will comment here only on some unexpected results (unpublished). We have roughly verified the fluorescence efficiency depletion when going from apohaemoglobins to haemoglobins, by comparing photon-counting rates in the same experimental conditions (globin counting rate $\sim 4 \times 10^4$ Hz; ferrihaemoglobin, $\sim 5 \times 10^2$ Hz, for example); lifetimes are, however, very little changed (the main component in the ferrihaemoglobin decay is only $\sim 20\%$ smaller: $\tau(\text{globin}) \sim 4.3$ ns, $\tau(\text{ferrihaemoglobin}) \sim 3.5$ ns, both at 3°C . (Apohaemoprotein and haemoprotein fluorescence decay are, in most cases, non-exponential, with the main component usually the shortest one.) Because our data are not refined, we cannot find significant changes in fluorescence decays using the three excitation wavelengths mentioned above.

To eliminate the possibility that the observed emission was not due to haem losses in the haemoprotein solution²¹, some samples were controlled by comparing the optical absorption in the 280-nm and 540-nm regions of their ferricyanomet derivatives. These controls have proved that the total amount of haem-free globin, if any, could not exceed 0.5%; a haem loss artefact can therefore be excluded.

An important decrease in the quantum yield, when fluorescence lifetimes are only slightly changed would indicate some sort of quenching with a strong 'static' component^{16,17}, the de-excitation being related to some non-fluorescent complex¹⁸ between the fluorophore (tryptophan) and the inhibitor (haem). It may be, however, that this non-fluorescent complex is not permanent, the static quenching indicating statistical fluctuations; the oscillations of tryptophan residues (it is known that the tryptophan emission in the globin is strongly depolarised, (unpublished results)) could correlate periodically, however, with the collective movements of the macromolecules in a particular orientation, and thus escape from the influence of haem. We could then imagine that, statistically, a small fraction ($\sim 1\%$) of the total excited tryptophan population are emitting their characteristic fluorescence. The small, but significant decrease in the ferrihaemoglobin (tetramer)

decay time, compared with that of the globin (dimer), could be explained by significant changes in the tryptophan microenvironment, reflecting global conformational changes induced in the macromolecule by the presence of haem.

On the $\alpha_{\text{SH}}\text{O}_2$ chain, besides the static quenching by the haem groups, there must also be a 'dynamic' one, because of the marked decrease both in the counting rate and in the lifetime: $\tau(\alpha \text{ globin}) \sim 4.4$ ns, $\tau(\alpha_{\text{SH}}\text{O}_2) \sim 1.0$ ns; both at 3°C (ref. 16). The $\beta_{\text{SH}}\text{O}_2$ chain shows a clear non-exponential decay, the main component having a lifetime of the order of 1.4 ns at 25°C ; the other one, much weaker, is much shorter (probably of the same order of magnitude as that of the α_{SH} chain, see below). The same behaviour is found in the case of ferrihaemoglobin with an O_2 ligand; here the main component of the decay is about the same (~ 1.5 ns; always at 25°C) but the contribution of the short component is much weaker than in the β chain. Note, however, that in the isolated α chain (monomer) there is a single tryptophan (tryp 14) residue; whereas in each subunit of the β protein (tetramer), there are two tryptophan residues (tryp 15 and 37). In addition, very little is known about the conformation of isolated α and β chains. Nevertheless there is a fundamental difference between the three haem proteins— $\alpha_{\text{SH}}\text{O}_2$, $\beta_{\text{SH}}\text{O}_2$ and HbO_2 —and the ferrihaemoglobin, that is, the absence of O_2 ligand in the latter.

We have also observed that lifetimes decrease substantially when increasing the temperature from 3 to 25°C : in the ferrihaemoglobin τ changes from ~ 3.5 ns to ~ 2.4 ns; from ~ 1.0 ns to ~ 0.5 ns, for the $\alpha_{\text{SH}}\text{O}_2$ chain. This time, in contrast with the unexpected results mentioned above, counting rates change in the same direction and in the same proportion, as would be expected. This behaviour could indicate substantial changes in the macromolecular conformation (it is known that the protein structure is very sensitive to temperature).

In addition, we must also consider a possible effect from oxygen; indeed, one could be tempted to attribute, at least partially, the dynamic quenching to oxygen diffusing into the protein^{18,19}. Although the macroscopic concentration of dissolved oxygen in the aqueous samples does not change very much in our temperature range ($\sim 3.9 \times 10^{-4}$ M at 3°C ; $\sim 2.5 \times 10^{-4}$ M, at 25°C), it is possible to imagine the presence of important local fluctuations: the intraprotein medium, covered with hydrophobic residues²⁰, could, in some conditions, act as an 'oxygen trap', the Henry laws being no longer valid. Another alternative hypothesis would be to consider the oxygen diffusion as being influenced by temperature-induced changes in the protein structure.

Further work will be necessary to answer some of these many questions—precise fluorescence quantum yield measurements, emission frequency distribution, time-resolved frequency distribution, and fluorescence time-dependent depolarisation.

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reviews

Images of terrestrial processes

Peter J. Smith

The Bowels of the Earth. By John Elder. Pp. vii+222. (Oxford University: London, 1976.) £6.00.

ACADEMIC writers have usually sought to develop as impersonal a style as possible, presumably in the belief that to do otherwise would be to impair their reputation for impartiality and objectivity or detract from the science itself. This tradition rightly persists, although at the same time there is a minor (but growing) and not entirely unwelcome movement towards the injection of 'personality' into the business. As far as the Earth sciences are concerned, the latter trend seems to have originated with Peter Wyllie (*The Dynamic Earth*, Wiley, 1971) who began in a small way by placing more overt emphasis on the scientists involved and later extended the technique with great effect in *The Way the Earth Works* (Wiley, 1976).

There is no doubt that in moderation, and performed with Wyllie's flair, an attempt to present a more sparkling view of science can be entertaining and give a more realistic picture of how science actually develops. But as Elder demonstrates here, it can also degenerate into irrelevance, absurdity and even crudity. The fact that the title of his book has achieved recognition in the new (1976) edition of *The Concise Oxford Dictionary* hardly disguises the vulgarity and gimmickry of its use in a serious context. It is not even accurate. How a word which refers metaphorically to 'innermost parts' can be taken to apply to the whole Earth from core to crust (not to mention the Earth as a body in space) is beyond imagining.

None of this would matter perhaps if it were not indicative of similar errors of judgement throughout the book, chiefly in the addition to inane metaphor and analogy. The Earth, for example, is variously a jam-pot, a stone, a jumping bean, an egg and much else besides, presumably in the mistaken impression that these homely analogies will appeal to what in these days of sexual equality Elder chooses to call 'the man in the street'. All of which is a pity because it obscures what is at heart a fascinating conception, namely, that the Earth may be viewed as a series of dynamic processes

occurring on a wide range of scales. To adapt one of his more sensible metaphors, Elder has given us an interesting sketchbook of the Earth—a collection of interacting models and images of terrestrial processes which vary in size, content, validity and credibility in reflection of what we know and can deduce from a body which is in the last resort inaccessible.

It is an unusual, if at times idiosyncratic, gallery which could be viewed with benefit by all Earth scientists, not least the 'experts' whom Elder explicitly excludes. But whether, as he fondly imagines, it is equally accessible to the

man in the street, I take leave to doubt; such an unfortunate creature would find himself bombarded by 'rather a lot of mathematical formulae' and is unlikely to find complete consolation in the index-cum-glossary. Some background in science would seem to me to be prerequisite. But with that reservation, and not forgetting the irritations and imperfections, I would recommend the book as an important way of looking at the terrestrial machine. □

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Molecular orbital theory

The Determination and Interpretation of Molecular Wave Functions. (Cambridge Monographs in Physical Chemistry, Vol. 3.) By Erich Steiner. Pp. viii+205. (Cambridge University: Cambridge and London, July 1976.) £11.50.

THERE are so many books on molecular orbital theory that it is hard to find a gap in the literature. One unfilled need is for a book at an elementary level which will bridge the separation between theoretical and experimental chemists. In particular such a book should enable the experimentalist to use the wave function computer programs which are so freely available thanks to the Quantum Chemistry Program Exchange. This book only fulfills this need in part.

The level is elementary and reasonably up to date, but the book contains no more than is to be found in a couple of chapters of standard texts supplemented by one or two recent review articles. Only molecular orbital theory is included. Indeed the preface states that "since 1960 there has been a declining importance of the valence bond approach as a practical tool of the computational quantum chemist"; a view which would not be supported by Goddard or the Italian or Bristol schools.

The early chapters summarise standard theory in a clear and concise manner, and introduce the required notation. Further chapters are devoted to the orbital approximation and be-

yond; to the representation of the orbitals and charge densities with a final look at the chemical bond. It is hard to quarrel with the content although the form in which it is presented reflects the author's view of the subject. Others might feel that natural orbitals, for example, are of interest less in a chapter on electron distribution than in the section on correlation energy.

Within the constraints of the book one definite omission is a detailed look at open-shell methods. So much published work is restricted to closed-shell ground states of molecules, possibly because a convenient account of the problems and techniques involved when there are unpaired electrons is not to be found in most simple texts. There is no coverage of molecular properties other than charge distribution.

For experimentalists an additional chapter on how to produce data for a standard computer program would have been helpful, together with some examples of how to enter these data and interpret the output. Without this, the book should be valuable as a reference work for the convetted who run calculations but probably not sufficient to enable the practical chemist to run his own. On the other hand, armed with this book he should appreciate what the theoretician has done.

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Access to soil ecology

The Distribution and Diversity of Soil Fauna. By John A. Wallwork. Pp. xii+355. (Academic: London and New York, April 1976.) £11; \$17.25.

IN 1970 Wallwork published his *Ecology of Soil Animals* which has since been accepted as an invaluable reference and teaching aid. With commendable frankness he introduces the new book as "something of an Adam's rib, a companion volume . . .". To anyone who has shared the development of his subject over the past thirty years, reading the new work is a most enjoyable experience for many original insights, a clear and often amusing style, and some superb illustrations. The atmosphere conveyed is almost that of a set of personal discussions, perhaps following a series of seminars, among people already familiar with the jargon and many of the ideas.

The chapters are mainly devoted to

major ecosystems in turn, grasslands, moorlands, forest soils and so on. Here Wallwork encounters the organisational problem which faces all writers of ecology books: the material to be covered is related in many logical 'dimensions' but must be discussed in linear order. Many topics, common to different ecosystems must either therefore be repeated or introduced rather arbitrarily in a particular chapter. The second practice is followed—for example, discussions of pedology and feeding biology in the grassland chapter—and the problem of re-locating a particular discussion is not fully met by means of cross references. One feels the need for a diagrammatic master plan in the introduction. The excellent index, however, goes a long way to overcome this difficulty.

The chapter on "agricultural practice and the soil fauna" is a short but valuable introduction to the literature of a fast-expanding field; and the final chapter faces the perennial problem of how so diverse a fauna persists in an

apparently rather uniform and narrow spatial zone. Throughout, the discussion is immensely strengthened by Wallwork's experience of soils from all parts of the world.

This is hardly, as claimed on the book jacket, "a basic introduction to the subject for students unfamiliar with the soil fauna". Many terms and ideas are either used without definition or are only defined after first introduction (the vocabulary of saprophage, fungivore, and so on, and the terms 'stentotopic' as against 'eurytopic'). These are perhaps further indications of the true readership of the book: it is probably best thought of as a synthesis, a companion volume and extension of *The Ecology of Soil Animals* and a valuable means of access to the literature of soil ecology.

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Somatic chromosomes

Chromosomes in Mitosis and Interphase. (Handbuch der Mikroskopischen Anatomie des Menschen.) By Hans George Schwarzscher. Pp. viii+182. (Springer: Berlin and New York, 1976.) DM 136; \$55.80.

THIS monograph is essentially concerned with the structure and organisation of the somatic chromosomes of man and I must say at once that I enjoyed reading it. Schwarzscher's excuse for writing such a book is the veritable flood of new information on human chromosomes that has been unleashed following the development and application of a wide variety of new techniques over the past few years.

The information and discussion is parcelled into ten chapters beginning with simple accounts of chromosome morphology, nomenclature and the human karyotype. There is a good review of what little we know of chromosome structure in the interphase nucleus as revealed by histochemical and electron microscope studies, supplemented by the observations on prematurely condensed chromosomes obtained by the method of Rao and Johnston using the cell fusion technique. Heterochromatin and chromosome fine structure, as studied by electron microscopy, are considered in detail, as are the main techniques utilised to produce the various banding patterns in human chromosomes. The two chapters on chromosome banding and fine structure are particularly

● *Climate and the Environment* by J. F. Griffiths (UK edition by Paul Elek reviewed in *Nature*, 262, August 5, 523, 1976) is also published in the USA by Westview Press, Boulder, Colorado 80301. Price: \$12.75.

good, and present a clear account of the facts and the various interpretations and speculations on mechanisms and structure. In such a rapidly developing field it is inevitable that some parts of the text are a little out of date—for example, some of the more recent work on human satellite DNAs is not discussed, but this is not a major criticism.

Geneticists who have not been following closely the human cytogenetic scene should be impressed by a wealth of chromosome polymorphisms that have been unearthed in the chromosome complements of various human populations using modern cytological techniques, and these are admirably summarised by the author. Man's chromosomes are varied indeed and, for example, our own studies in Edinburgh reveal that some 3% of the local population have inversions involving the whole or part of the C band on chromosome 1; that all of us are heteromorphic for one or more C- or Q-band regions; and that the chromosome phenotype of an individual may be almost as distinctive as his fingerprints! Schwarzscher, in fact, points out that an analysis of chromosome polymorphisms would give successful paternity exclusion tests in about 75% or more of cases.

The text of the book is very clearly written; it is well illustrated and easy

to read with only rare glimpses of minor problems of translation from the German, as in sentences such as "With this term the morphologic separation of complete chromosome sets of genomes is meant."

The general style tends to be rather more descriptive than discursive and I personally would have preferred more discussion of some of the interesting points brought out by the author. For example, a photograph and two lines of text impart the information—substantiated by all workers interested in chromosome replication—that daughter strands are always placed to the outside of parental templates. This implies a certain pattern of DNA segregation which must of itself reflect on organisation of chromosome structure—something that is not really considered in the book.

This little book is timely, and shows that we have come quite a long way in the past five years or so towards revealing the enormous diversity and variation in structure of man's chromosomes, but we have a long way yet to travel before we can really understand what these variations mean both in terms of chromosome organisation and in possible consequences to the individual. Schwarzscher's summary sets a very useful background and is to be highly recommended to those who are already journeying in this field or who are about to start on their travels.

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obituary

Dr A. P. Rowe, the wartime Head of the Telecommunications Research Establishment—the home of radar—died at Malvern in May 1976 at the age of 78.

Albert Percival Rowe trained as a meteorologist and worked in the Air Ministry's Laboratory in Imperial College after the 1914–18 War. From there he became Personal Assistant to H. E. Wimperis, the first Director of Scientific Research at the Air Ministry; and in June 1934—alarmed by the inadequacy of air defence in Britain, as he related in his book *One Story of Radar* (1948)—he warned the Air Ministry that “Unless science evolved some new method of aiding air defence, we were likely to lose the next war if it started within ten years”. In the following November, Wimperis proposed the formation of a Committee for the Scientific Survey of Air Defence, which quickly took shape as the famous Tizard Committee.

The story has been told many times of how the principle of radar was outlined by Watson-Watt to the Committee at its first meeting, as a result of a query about death rays put to him by Wimperis, and how the independent approach of F. A. Lindemann and Churchill led to intense friction. Rowe's part in these events was to be the first Secretary of the Tizard Committee, and to decide with Wimperis to buy Bawdsey Manor, just north of Felixstowe, to house the research and development work that would be needed before radar could be made operational. In the spring of 1938 Rowe succeeded Watson-Watt as Superintendent of what was then known as the Air Ministry Research Establishment, Bawdsey, and he remained Superintendent, later Chief Superintendent, of this Establishment and its successors until the end of the war, by which time it was the great Telecommunications Research Establishment at Malvern with a staff of 3,000.

The basic notion of radar came from a Government establishment, the Radio Research Station of the National Physical Laboratory, staffed by men who—with Rowe—had gone into Government service between the wars rather than into universities. Great though the contributions of the latter were subsequently to be, they would mainly have come too late for the Battle of Britain, and it was our national good fortune that there was enough talent in Government service to

enable us to prepare for that critical phase. Recruiting from the universities had started in 1935, with men such as E. G. Bowen, R. Hanbury Brown, and A. G. Touch; and it was these recruits who, together with Watson-Watt's original nucleus of L. H. Bainbridge Bell and A. F. Wilkins, did the essential early work. Academic reluctance to work on military projects was dispelled by the Anschluss of 1938, and by the outbreak of war there was a flood of willing—and distinguished—scientific recruits, most of whom went into radar.

Rowe therefore found himself at the head of a great body of talent, from which ideas and inventions flowed throughout the war. Somehow he had to guide his establishment of scientists and engineers, few of them accustomed to Service or Civil Service procedures, into effective relationships both with the Royal Air Force and with industry. Throughout the war he held vigorously the belief that the work his Establishment was doing was vital; and at the end of it he wrote “We believe that without radar Fighter Command and the ‘famous few’ would have lost the Battle of Britain. We believed that without radar the night attacks by enemy bombers would have devastated our industries. We believed that without radar the work and gallantry of Bomber Command would have been largely wasted; and we believed that without radar the sea-war would have been lost and that there would have been no invasion of Europe”. Although by 1948 he had begun to wonder whether this ‘overwhelming belief’ had been entirely justified, it had been his guiding star throughout the war and he would declare it to his staff and outsiders alike.

He was completely sincere, and it was his sincerity that enabled him to keep control of what could easily have been a very unruly Establishment. Sometimes this same sincerity led him into slightly bizarre situations: on one occasion during the war he sent a circular letter to the wives of his staff telling them that their husbands were doing work of the utmost national importance but that, unlike the serving officers with whom they were working, they were entitled only to ordinary civilian rations. Since there were various deficiencies in wartime food, it was essential that each wife should do her best to supplement her husband's diet, notably in respect of vitamins and

vitamin B in particular. This vitamin was to be had in abundance in ‘Bemax’ and so would she please feed this to her husband every morning. In a similar vein he had issued a station instruction at Bawdsey pointing out that the dispersal of the several research teams in huts hundreds of yards apart inhibited communication and the cross-fertilisation of ideas: he therefore urged all members of the staff to buy second-hand bicycles, and added the address of a cycle dealer in Felixstowe whom he had ascertained to have an adequate supply. But despite the comic effect produced by such instructions, he was obviously so genuine that he kept the respect, which with the years developed into affection, of his staff.

Perhaps his greatest individual contribution was the ‘Sunday Soviet’ held weekly in his room which, after the move of TRE to Malvern College, was of course the Headmaster's Study. Sundays were convenient for such men as Lord Cherwell, Sir Robert Renwick, and senior serving officers to visit Malvern, and Rowe would gather members of his staff for a free-for-all discussion which would last from morning well into the afternoon. Bernard Lovell, then one of Rowe's men, has recorded that “A Commander-in-Chief once complained in Rowe's office that the most lowly assistant in the Establishment would not turn a screw unless he could be acquainted with the latest strategic situation in the Middle East”. The interplay between scientists and serving officers had not been started by Rowe; from their experiences in the 1914–18 War, Lindemann and Tizard, for all their differences, were united in the importance of this interplay, but it reached one of its highest expressions in Rowe's Soviets. These meetings were one of the main factors in the ultimate superiority of British radar over its counterpart in Germany, where scientists and military men were not nearly in such effective contact.

The successes of TRE were many. As regards actual inventiveness, the ideas of course originated with individuals, to whom full credit should be given; and the characteristic problems associated with getting individuals to work in a corporate body are probably less in war than in peace, where the importance and urgency of a common aim are less clear. But, even so, with all the brilliance of individuals and the corporate spirit engendered by national emergency, TRE would have been far

less effective if it had not been so well guided, and this was Rowe's contribution. He would certainly have been blamed had TRE failed, and in its success he deserved a greater honour than the CBE that was awarded to him in 1942. In fact, the whole of the radar effort was miserably recognised during the war: the Americans, who were glad to have a contingent at TRE, gave Rowe a more fitting recognition—the Medal for Merit, their highest civilian decoration, awarded directly by the President.

Writing to me in 1974 of such matters he said "I have never written of those things to anyone till now. I feel strongly about two lines of a prayer, 'To toil and not to seek for rest, To labour and not to seek for any reward'. Certainly I never thought of it during the war, when vast numbers were giving their lives".

After the war, Rowe first became the Deputy Controller of Research and Development at the Admiralty, and then in 1947 went to Australia as Chairman of the Defence Advisory Committee and Defence Scientific Adviser to the Australian Government, and in 1948 he became Vice-Chancellor of the University of Adelaide. This experience was not a happy one, as Rowe himself related in his book *If*

the Gown Fits (1960). Clearly he found the university a far less ready body to accept his guidance than TRE had been. He thought that universities should be more down-to-earth than they were in meeting the needs of the average rather than the brightest student; but, almost paradoxically, he concluded "It is necessary to replace fearfulness by courage, departmentalism by unity of purpose, egalitarianism by a measure of authority, and to recognise excellence wherever it rears its noble head". And if Australian universities are now better than they were thirty years ago, part of the change may be due to the unpopular stand that he took.

Rowe had never been afraid of expressing an unpopular view, but he felt the unpopularity all the same, when it led to loneliness as in Australia. It was here that he valued especially the support of his wife, Mary, whom he married in 1932: "Far from family and friends" he wrote in *If the Gown Fits*, "she unfalteringly supported me in my years of unaccustomed loneliness".

He came back to Malvern in 1958 and spent some of his later years teaching astronomy to the boys of the College. But—such is the way that a nation sometimes treats its faithful servants—he found it so difficult to live

on his pension that he actually left Malvern again for Malta, where the cost of living was lower. We were glad to see him back again when conditions in Malta proved not to be what he had hoped for. He had the idea of writing a further book, on invention in war, but he found the work too much and handed his papers over to Guy Hartcup, who produced the book as *The Challenge of War* (1970).

When in 1974 the Royal Society organised a Symposium on *The Effects of the Two World Wars on the Development and Organisation of Science in the United Kingdom*, Rowe was unable to attend. The meeting had the atmosphere of a reunion, and it spontaneously sent him a telegram of tribute signed by many of his wartime colleagues headed by the President, Alan Hodgkin, who as a member of TRE himself had made the first flight with airborne centimetric radar in 1941.

His final tribute came on June 18, 1976, when as many of his former colleagues as possible gathered in Malvern Priory for his Memorial Service and to recall his vital contribution as the head of what J. A. Ratcliffe in his Oration fairly described as "one of the most successful research establishments of all time".

R. V. Jones

announcements

Appointments

Dr B. J. Mason as President of the Institute of Physics, and Dr G. H. Stafford as Vice-President.

Awards

The Royal Society has given the S. G. Brown Award and Medal to **Frank Mackley**, a Director and Chief Engineer of J. T. Mackley & Co. Ltd, for his work on the development of the Hover Platform.

The John Scott Award for 1976 has been made to **Professor Cyril A. Clarke** and **Drs Vincent J. Freda, John G. Gorman and William Pollack** for their work on the prevention of Rhesus Haemolytic Disease.

Meetings

October 29, **Hybrids in Botany, Horticulture and Agriculture**, Leicester (Dr C. A. Stace, Botanical Laboratories, The University, Leicester LE1 7RH, UK).

November 15–18, **Weeds**, Brighton, Sussex (Mr W. F. P. Bishop, Frank Bishop (Conference Planners Ltd), 74

Person to Person

A survey is being initiated into the distribution and types of symbiotic *Chlorella* sp. in Britain. Information is needed on precise locations of natural habitats of green Hydra, *Paramecium bursaria*, *Stentor*, Sponges and other invertebrates definitely containing *Chlorella*. Please write to D. C. Smith, Department of Botany, The University, Bristol BS8 1UG.

Professor and wife wish to find small, furnished flat in S. Kensington or Belgravia, Dec. 15–30, 1976. Will exchange fully furnished 3-bedroomed house in Georgetown, Washington, D.C., Dec. 14–Jan. 6. Address replies and enquiries to D. N. Robinson, 1237 37th Street, N.W., Washington, D.C. 20007.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

London Road, Croydon CR0 2TB).

November 24, **Whole Body Counting and Scanning**, London (The General Secretary, British Institute of Radiology, 32 Welbeck Street, London W1M 7PG).

December 12–17, **Automated Cytology**, Pensacola, Florida (Paul F. Mullaney, Chairman, Fifth Engineering Foundation Conference on Automated Cytology, PO Box 208, Los Alamos, New Mexico 87544).

April 14–15, 1977, **Easter Meeting of the Society for the Bibliography of Natural History**, London (Meetings Secretary, Mrs J. A. Diment, Palaeontology Library, British Museum (Natural History), Cromwell Road, London SW7 5BD).

April 18–20, 1977, **Stem Cells and Tissue Homeostasis**, Manchester (Dr R. J. Cole, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG).

May 9–11, 1977, **Biosystematics in Agriculture**, Beltsville, Maryland (Dr James A. Duke, Publicity Committee, BARC Symposium II, Plant Taxonomy Laboratory, Room 117, Building 001, BARC West, USDA, Beltsville, Maryland 20705).